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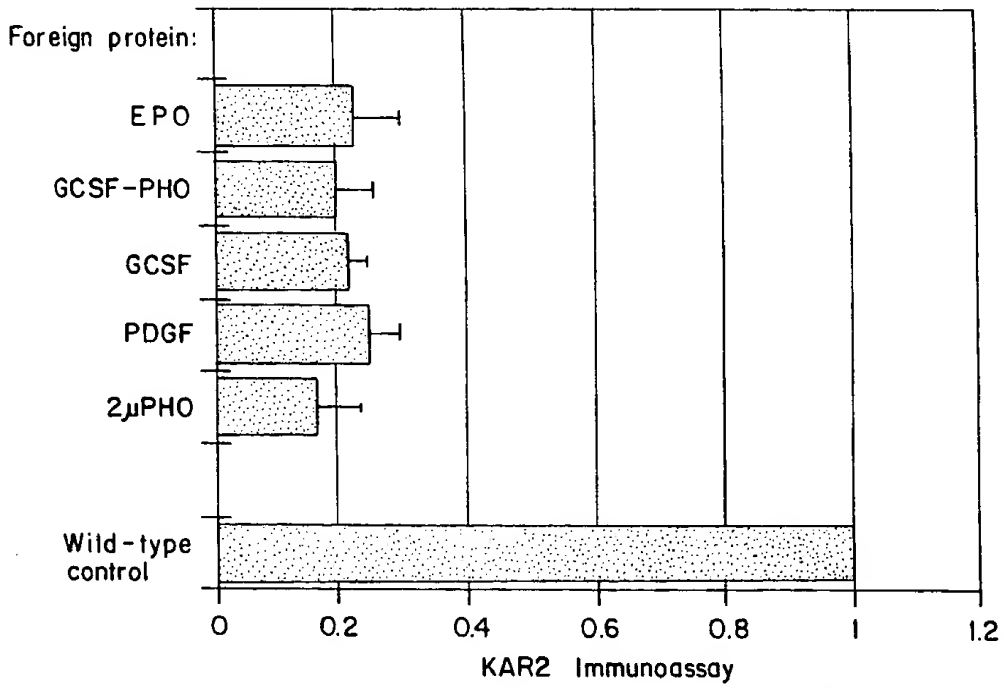

(57) Abstract

The present invention is directed to methods for increasing secretion of an overexpressed gene product present in a host cell, by inducing expression of chaperone proteins within the host cell.

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METHODS FOR INCREASING SECRETION OF OVEREXPRESSED PROTEINS

The present invention relates to methods for increasing protein secretion of overexpressed gene products by enhancing chaperone protein expression within a host cell. Chaperone proteins which can increase protein secretion include protein folding chaperone proteins which bind to and assist in the folding of unfolded polypeptides. Such protein folding chaperone proteins include heat shock protein 70 (hsp70) class of proteins such as mammalian or yeast HSP68, HSP70, HSP72, HSP73, clathrin uncoating ATPase, IgG heavy chain binding protein (BiP), glucose-regulated proteins 75, 78 and 80 (GRP75, GRP78 and GRP80), HSC70, and yeast KAR2, BiP, SSA1-4, SSB1, SSD1 and the like. Chaperone proteins which can increase protein secretion also include enzymes which catalyze covalent modification of proteins, such as mammalian or yeast protein disulfide isomerase (PDI), prolyl-4-hydroxylase ß-subunit, ERp59, glycosylation site binding protein (GSBP) and thyroid hormone binding protein (T3BP).

Many proteins can be reversibly unfolded and refolded *in vitro* at dilute concentrations since all of the information required to specify a compact folded protein structure is present in the amino acid sequence of a protein. However, protein folding *in vivo* occurs in a concentrated milieu of numerous proteins in which intermolecular aggregation reactions compete with the intramolecular folding process.

Moreover, gene products which are highly overexpressed are often poorly secreted even though secretion signals are present on such overexpressed gene products (Biemans et al. 1991 DNA Cell Biol. 10: 191-200; Elliot et al. 1989 Gene 79: 167-180; and Moir et al. 1987 Gene 56: 209-217). The prior art has not provided a clear reason for, or a simple and efficient means to overcome, such poor secretion of overexpressed gene products.

Recently, a class of proteins have been identified which are associated with the intracellular folding of nascently formed polypeptides. Such proteins have been named 'chaperone' proteins (e.g. see reviews by Ellis et al. 1991 Annu. Rev. Biochem. 60: 321-347; Gething et al. (1992) Nature 355: 33-45; Rothman 1989 Cell 59: 591-601; Horwich et al. 1990 TIBTECH 8: 126-131; and Morimoto et al. (Eds.) 1990 Stress Proteins in Biology and Medicine, Cold Spring Harbor Press: Cold Spring Harbor, NY, pp. 1-450).

At least two classes of chaperone proteins are involved in polypeptide folding in cells. Enzymes such as protein disulfide isomerase (PDI) and peptidyl prolyl isomerase (PPI) can covalently modify proteins by catalyzing specific isomerization steps that may limit the folding rate of some proteins. (Freedman, R.B. 1989 Cell 57: 1067-1072). Another type of chaperone binds to folding intermediates but not to folded proteins and apparently causes no covalent modification of such intermediates. This latter type is referred to herein as a protein folding chaperone.

Chaperone proteins that can covalently modify proteins include PDI and PPI. PDI catalyzes

thiol/disulfide interchange reactions and promotes disulfide formation, isomerization or reduction, thereby facilitating the formation of the correct disulfide pairings, and may have a more general role in the prevention of premature misfolding of newly translocated chains.

PDI interacts directly with newly synthesized secretory proteins and is required for the folding of nascent polypeptides in the endoplasmic reticulum (ER) of eukaryotic cells. Enzymes found in the ER with PDI activity include mammalian PDI (Edman et al., 1985, Nature 317:267), yeast PDI (Mizunaga et al. 1990, J. Biochem. 108:848), mammalian ERp59 (Mazzarella et al., 1990, J. Biochem. 265:1094), mammalian prolyl-4-hydroxylase (Pihlajaniemi et al., 1987, EMBO J. 6: 643) yeast GSBP (Lamantia et al., 1991, Proc. Natl. Acad. Sci. USA, 88:4453) and mammalian T3BP (Yamauchi et al., 1987, Biochem. Biophys. Res. Commun. 146:1485), and yeast EUG1 (Tachibana et al., 1992, Mol. Cell Biol. 12, 4601).

Two major families of protein folding chaperones have been identified, a heat shock protein 60 (hsp60) class and a heat shock protein 70 (hsp70) class. Chaperones of the hsp60 class are structurally distinct from chaperones of the hsp70 class. In particular, hsp60 chaperones appear to form a stable scaffold of two heptamer rings stacked one atop another which interacts with partially folded elements of secondary structure (Ellis et al. 1991; and Landry et al. 1992 Nature 355: 455-457). On the other hand, hsp70 chaperones are monomers or dimers and appear to interact with short extended regions of a polypeptide (Freiden et al. 1992

EMBO J. 11: 63-70; and Landry et al. 1992). Hsp70 and hsp60 chaperones may also have sequential and complementary protein folding roles wherein hsp70 proteins bind to extended polypeptide chains to prevent aggregation and hsp60 oligomers complete the folding of the extended polypeptide chain (Langer et al. 1992 Nature 354: 683-689).

While hsp60 homologs appear to exist mainly within mitochondria and chloroplasts of eukaryotic cells, most compartments of eukaryotic cells contain members of the hsp70 class of chaperones. A eukaryotic hsp70 homolog originally identified as the IgG heavy chain binding protein (BiP) is now known to have a more general role in associating with misfolded, unassembled or aberrantly glycosylated proteins. BiP is located in all eukaryotic cells within the lumen of the endoplasmic reticulum (ER). BiP is a soluble protein which is retained in the ER by a receptor-mediated recycling pathway and perhaps by calcium crosslinking (Pelham 1989 Annu. Rev. Cell. Biol. 5: 1-23; Sambrook 1990 Cell 61: 197-199).

Hsp70 chaperones are well conserved in sequence and function (Morimoto et al. 1990). For example, the DnaK hsp70 protein chaperone in Escherichia coli, shares about 50% sequence homology with an hsp70 KAR2 chaperone in yeast (Rose et al. 1989 Cell 57:1211-1221). Moreover, the presence of mouse BiP in yeast can functionally replace a lost yeast KAR2 gene (Normington et al. 19: 1223-1236). Such a high structural and functional conservation for BiP has led to a generic usage for the term BiP as meaning any protein folding chaperone which resides in the endoplasmic reticulum of eukaryotes ranging from yeast to humans.

The first step in the eukaryotic secretory pathway is translocation of the nascent polyp ptide across the ER membrane in extended form. Correct folding and assembly of a polypeptide occurs in the ER and is a prerequisite for transport from the ER through the secretory pathway (Pelham 1989 Annu. Rev. Cell. Biol. 5: 1-23; Gething et al. 1990 Curr. Op. Cell Biol. 1: 65-72). For example, translocation intermediates which are artificially lodged in microsomal membranes in vitro can be chemically crosslinked with BiP (Sanders et al. 1992 Cell 69: 354-365). Therefore, misfolded proteins are retained in the ER, often in association with BiP (Suzuki et al. 1991 J. Cell Biol. 114: 189-205).

The association of chaperone proteins with misfolded proteins has led some workers to conclude that hsp70 chaperone proteins like BiP act as proofreading proteins, whose chief role is to bind to and prevent secretion of misfolded proteins (Dorner et al. 1988 J. Mol. & Cell. Biol. 8:4063-4070; Dorner et al. 1992 EMBO J. 11: 1563-1571). Dorner et al. (1992) have also suggested that overexpression of the BiP hsp70 chaperone protein can actually block secretion of selected proteins in Chinese hamster ovary cells. Therefore, according to the prior art, the role of BiP is to inhibit protein secretion.

In contrast, the present invention provides methods for increasing protein secretion, unexpectedly, by increasing expression of an hsp70 chaperone protein or a PDI chaperone protein. Moreover, according to the present invention, it has been discovered that soluble forms of PDI and hsp70 chaperone protein are diminished in cells which have been caused to overexpress a gene product. Therefore, the present methods can be used for

increasing protein secretion by circumventing this dimunition of PDI and/or hsp70 chaperone protein expression.

The present invention provides a method for increasing secretion of overexpressed gene products from a host cell, which comprises expressing at least one chaperone protein in the host cell. In the present context, an overexpressed gene product is one which is expressed at levels greater than normal endogenous expression for that gene product. Overexpression can be effected, for example, by introduction of a recombinant construction that directs expression of a gene product in a host cell, or by altering basal levels of expression of an endogenous gene product, for example, by inducing its transcription.

In one embodiment, the method of the invention comprises effecting the expression of at least one chaperone protein and an overexpressed gene product in a host cell, and cultivating said host cell under conditions suitable for secretion of the overexpressed gene product. The expression of the chaperone protein and the overexpressed gene product can be effected by inducing expression of a nucleic acid encoding the chaperone protein and a nucleic acid encoding the overexpressed gene product wherein said nucleic acids are present in a host cell. In another embodiment, the expression of the chaperone protein and the overexpressed gene product are effected by introducing a first nucleic acid encoding a chaperone protein and a second nucleic acid encoding a gene product to be overexpressed into a host cell under conditions suitable for expression of the first and second nucleic acids.

In a preferred embodiment, one or both of said first and second nucleic acids are present in expression vectors.

In another embodiment, expression of said chaperone protein is effected by inducing expression of a nucleic acid encoding said chaperone protein wherein said nucleic acid is present in a host cell or by introducing a nucleic acid encoding said chaperone protein into a host cell. Expression of said second protein is effected by inducing expression of a nucleic acid encoding said gene product to be overexpressed wherein said nucleic acid is present in a host cell or by introducing a nucleic acid encoding said second gene product into the host cell.

In a preferred embodiment, the host cell is a yeast cell or a mammalian cell.

In another preferred embodiment, the chaperone protein is an hsp70 chaperone protein or a protein disulfide isomerase. The hsp70 chaperone protein is preferably yeast KAR2 or mammalian BiP. The protein disulfide isomerase is preferably yeast PDI or mammalian PDI.

The present invention further provides a method for increasing secretion of an overexpressed gene product in a yeast host cell by using a yeast KAR2 chaperone protein, or yeast PDI, or yeast KAR2 in combination with yeast PDI, in the present methods.

The present invention also provides a method for increasing secretion of an overexpressed gene product in a mammalian host cell by using a mammalian BiP chaperone protein, or mammalian PDI, or mammalian BiP in combination with mammalian PDI, in the present methods.

Fig. 1 depicts the amounts of soluble KAR2 protein present in cell extracts of wild type yeast and yeast strains overexpressing human erythropoietin (EPO), human platelet derived growth factor B chain (PDGF), human granulocyte colony stimulating factor (GCSF), Schizosaccharomyces pombe acid phosphatase (PHO) and a fusion between GCSF and PHO (GCSF-PHO) in a constitutive manner.

Fig. 2 depicts a pMR1341 expression vector which contains the yeast KAR2 gene. As depicted, this vector encodes ampicillin resistance (Amp^R), a pSC101 origin of replication (ori pSC101), a CEN4 centromeric sequence, an ARS1 autonomous replication sequence, a URA3 selectable marker and the PGAL1 promoter is used to effect expression of the KAR2 chaperone protein. In other experiments the URA3 selectable marker was deleted and replaced with HIS and LEU selectable markers.

Fig. 3 depicts the KAR2 expression observed in cell extracts collected from wild type cells (•), cells transformed with the EPO-encoding plasmid only (▪, GalEpo) and cells transformed with both the EPO-encoding plasmid and the KAR2-encoding plasmid (▲, GalEpo+GalKar2) at 24, 48 and 72 hours after induction of KAR2 and EPO expression.

Fig. 4 depicts the growth of wild type cells (□), cells transformed with the EPO-encoding plasmid only (o, GalEpo) and cells transformed with both the EPO-encoding plasmid and the KAR2-encoding plasmid (Δ, GalEpo+GalKar2). The inset provided in Fig. 4 depicts the amount of EPO secreted into the medium of cells having the EPO-encoding plasmid only (GalEpo) compared with the amount of secreted EPO for cells having both the EPO-encoding plasmid and the KAR2-encoding plasmid

(GalEpo + GalKar2) during exponential growth of these yeast strains at the indicated time point (arrow).

According to the present invention, it has been discovered that the amount of chaperone proteins can be diminished in cells during overexpression of a gene product and this diminution in chaperone protein levels can lead to depressed protein secretion. Moreover, in accordance with the present invention it has been found that an increase in chaperone protein expression can increase secretion of an overexpressed gene product.

Therefore, the present invention relates to a method for increasing secretion of an overexpressed gene product present in a host cell, which includes expressing a chaperone protein in the host cell and thereby increasing secretion of the overexpressed gene product.

The present invention also contemplates a method of increasing secretion of an overexpressed gene product from a host cell by expressing a chaperone protein encoded by an expression vector present in or provided to the host cell, thereby increasing the secretion of the overexpressed gene product.

The present invention provides a method for increasing secretion of overexpressed gene products from a host cell, which comprises expressing at least one chaperone protein in the host cell. In the present context, an overexpressed gene product is one which is expressed at levels greater than normal endogenous expression for that gene product. Overexpression can be effected, for example, by introduction of a recombinant construction that directs expression of a gene product

in a host cell, or by altering basal levels of expression of an endogenous gene product, for example, by inducing its transcription.

In one embodiment, the method of the invention comprises effecting the expression of at least one chaperone protein and an overexpressed gene product in a host cell, and cultivating said host cell under conditions suitable for secretion of the overexpressed gene product. The expression of the chaperone protein and the overexpressed gene product can be effected by inducing expression of a nucleic acid encoding the chaperone protein and a nucleic acid encoding the overexpressed gene product wherein said nucleic acids are present in a host cell.

In another embodiment, the expression of the chaperone protein and the overexpressed gene product are effected by introducing a first nucleic acid encoding a chaperone protein and a second nucleic acid encoding a gene product to be overexpressed into a host cell under conditions suitable for expression of the first and second nucleic acids. In a preferred embodiment, one or both of said first and second nucleic acids are present in expression vectors.

In another embodiment, expression of said chaperone protein is effected by inducing expression of a nucleic acid encoding said chaperone protein wherein said nucleic acid is present in a host cell or by introducing a nucleic acid encoding said chaperone protein into a host cell. Expression of said second protein is effected by inducing expression of a nucleic acid encoding said gene product to be overexpressed wherein said nucleic acid is present in a host cell or by introducing a nucleic acid encoding said second gene product into the host cell.

In a preferred embodiment, the host cell is a yeast cell or a mammalian cell.

In another preferred embodiment, the chap rone protein is an hsp70 chaperone protein or a protein disulfide isomerase. The hsp70 chaperone protein is preferably yeast KAR2 or mammalian BiP. The protein disulfide isomerase is preferably yeast PDI or mammalian PDI.

The present invention further provides a method for increasing secretion of an overexpressed gene product in a yeast host cell by using a yeast KAR2 chaperone protein, or yeast PDI, or yeast KAR2 in combination with yeast PDI, in the present methods.

The present invention also provides a method for increasing secretion of an overexpressed gene product in a mammalian host cell by using a mammalian BiP chaperone protein, or mammalian PDI, or mammalian BiP in combination with mammalian PDI, in the present methods.

Chaperone proteins of the present invention include any chaperone protein which can facilitate or increase the secretion of proteins. In particular, members of the protein disulfide isomerase and heat shock 70 (hsp70) families of proteins are contemplated. An uncapitalized "hsp70" is used herein to designate the heat shock protein 70 family of proteins which share structural and functional similarity and whose expression are generally induced by stress. To distinguish the hsp70 family of proteins from the single heat shock protein of a species which has a molecular weight of about 70,000, and which has an art-recognized name of heat shock protein-70, a capitalized HSP70 is used herein. Accordingly, each member of the hsp70

family of proteins from a given species has structural similarity to the HSP70 protein from that species.

The present invention is directed to any chaperone protein having the capability to stimulate secretion of an overexpressed gene product. The members of the hsp70 family of proteins are known to be structurally homologous. Moreover, according to the present invention any hsp70 chaperone protein having sufficient homology to the KAR2 polypeptide sequence can be used in the present methods to stimulate secretion of an overexpressed gene product. Members of the PDI family are also structurally homologous, and any PDI which can be used according to the present method is contemplated herein. In particular, mammalian and yeast PDI, prolyl-4-hydroxylase ß-subunit, ERp59, GSBP and T3BP and yeast EUG1 are contemplated.

As used herein, homology between polypeptide sequences is the degree of colinear similarity or identity between amino acids in one polypeptide sequence with that in another polypeptide sequence. Hence, homology can sometimes be conveniently described by the percentage, i.e. proportion, of identical amino acids in the sequences of the two polypeptides. For the present invention sufficient homology means that a sufficient percentage of sequence identity exists between an hsp70 chaperone polypeptide sequence and the KAR2 polypeptide sequence of SEQ ID NO:2, or between a PDI protein and the yeast PDI polypeptide sequence of SEQ ID NO:18 or the mammalian PDI sequence of SEQ ID NO:20 to retain the requisite function of the chaperone protein, i.e. stimulation of secretion.

Therefore a sufficient number, but not necessarily all, of the amino acids in the present hsp70 chaperone polypeptide sequences are identical to the

KAR2 polypeptide sequence of SEQ ID NO:2, or the yeast PDI polypeptide sequence of SEQ ID NO:18 or the mammalian PDI polypeptide of SEQ ID NO:20. In particular, the degree of homology between an hsp70 chaperone protein of the present invention and the polypeptide sequence of SEQ ID NO:2 need not be 100% so long as the chaperone protein can stimulate a detectable amount of gene product secretion. However, it is preferred that the present hsp70 chaperone proteins have at least about 50% homology with the polypeptide sequence of SEQ ID NO:2. In an especially preferred embodiment sufficient homology is greater than 60% homology with the KAR2 polypeptide sequence of SEQ ID NO:2. Similarly, the degree of homology between a PDI chaperone protein and the polypeptide sequence or SEQ ID NO:18 or 20 need not be 100% so long as the chaperone protein can stimulate a detectable amount of a gene product secretion. At least about 50% homology is preferred.

The number of positions which are necessary to provide sufficient homology to KAR2 or PDI to retain the ability to stimulate secretion can be assessed by standard procedures for testing whether a chaperone protein of a given sequence can stimulate secretion.

Procedures for observing whether an overexpressed gene product is secreted are readily available to the skilled artisan. For example, Goeddel, D.V. (Ed.) 1990, Gene Expression Technology, Methods in Enzymology, Vol 185, Academic Press, and Sambrook et al. 1989, Molecular Cloning: A Laboratory Manual, Vols. 1-3, Cold Spring Harbor Press, N.Y., provide procedures for detecting secreted gene products.

To secrete an overexpressed gene product the host cell is cultivated under conditions sufficient for

secretion of the overexpressed gene product. Such conditions include temperature, nutri nt and cell density conditions that permit secretion by the cell. Moreover, such conditions are conditions under which the cell can perform basic cellular functions of transcription, translation and passage of proteins from one cellular compartment to another and are known to the skilled artisan.

Moreover, as is known to the skilled artisan a secreted gene product can be detected in the culture medium used to maintain or grow the present host cells. The culture medium can be separated from the host cells by known procedures, e.g. centrifugation or filtration. The overexpressed gene product can then be detected in the cell-free culture medium by taking advantage of known properties characteristic of the overexpressed gene product. Such properties can include the distinct immunological, enzymatic or physical properties of the overexpressed gene product.

For example, if an overexpressed gene product has a unique enzyme activity an assay for that activity can be performed on the culture medium used by the host cells. Moreover, when antibodies reactive against a given overexpressed gene product are available, such antibodies can be used to detect the gene product in any known immunological assay (e.g. as in Harlowe, et al., 1988, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press).

The secreted gene product can also be detected using tests that distinguish proteins on the basis of characteristic physical properties such as molecular weight. To detect the physical properties of the gene product all proteins newly synthesized by the host cell can be labeled, e.g. with a radioisotope. Common

radioisotopes which are used to label proteins synthesized within a host cell include tritium (^{3}H), carbon-14 (^{14}C), sulfur-35 (^{35}S) and the like. For example, the host cell can be grown in ^{35}S-methionine or ^{35}S-cysteine medium, and a significant amount of the ^{35}S label will be preferentially incorporated into any newly synthesized protein, including the overexpressed protein. The ^{35}S containing culture medium is then removed and the cells are washed and placed in fresh non-radioactive culture medium. After the cells are maintained in the fresh medium for a time and under conditions sufficient to allow secretion of the ^{35}S radiolabelled overexpressed protein, the culture medium is collected and separated from the host cells. The molecular weight of the secreted labeled protein in the culture medium can then be determined by known procedures, e.g. polyacrylamide gel electrophoresis. Such procedures are described in more detail within Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Vols. 1-3, Cold Spring Harbor Press, NY).

Thus for the present invention, one of ordinary skill in the art can readily ascertain which chaperone proteins have sufficient homology to KAR2 or PDI to stimulate secretion of an overexpressed gene product.

According to the present invention, hsp70 chaperone proteins include yeast KAR2, HSP70, BiP, SSA1-4, SSB1, SSC1 and SSD1 gene products and eukaryotic hsp70 proteins such as HSP68, HSP72, HSP73, HSC70, clathrin uncoating ATPase, IgG heavy chain binding protein (BiP), glucose-regulated proteins 75, 78 and 80 (GRP75, GRP78 and GRP80) and the like.

Preferred PDI chaperone proteins include yeast and mammalian PDI, mammalian ERp59, mammalian prolyl-4-

hydroxylase B-subunit, yeast GSBP, yeast EUG1 and mammalian T3BP.

Preferred chaperone proteins of the present invention normally reside within the endoplasmic reticulum of the host cell. For example, chaperone proteins which are localized with the endoplasmic reticulum include KAR2, GRP78, BiP, PDI and similar proteins.

Moreover, the polypeptide sequence for the present hsp70 chaperones preferably has at least 50% sequence homology with a yeast KAR2 polypeptide sequence having SEQ ID NO:2. The hsp70 chaperone polypeptide sequences which have at least 50% sequence homology with SEQ ID NO:2 include, for example, any yeast HSP70, BiP, SSD1 and any mammalian or avian GRP78, HSP70 or HSC70.

Preferred hsp70 chaperone polypeptide sequences include, for example:

Saccharomyces cerevisiae KAR2 having a nucleotide sequence corresponding to SEQ ID NO:1 and a polypeptide sequence corresponding to SEQ ID NO:2 (Rose et al. 1989 Cell 57: 1211-1221; Normington et al. 1989 Cell 57: 1223-1236);

Schizosaccharomyces pombe HSP70 having a nucleotide sequence corresponding to SEQ ID NO:3 and a polypeptide sequence corresponding to SEQ ID NO:4 (Powell et al. 1990 Gene 95:105-110);

Kluyveromyces lactis BiP having a polypeptide sequence corresponding to SEQ ID NO:5 (Lewis et al. 1990 Nucleic Acids Res. 18: 6438);

Schizosaccharomyces pombe BiP having a nucleotide sequence corresponding to SEQ ID NO:6 and a polypeptide sequence corresponding to SEQ ID NO:7 (Pidoux et al. 1992 EMBO J. 11: 1583-1591);

Saccharomyces cerevisiae SSD1 having a nucleotide sequence corresponding to SEQ ID NO:8 and a polypeptide sequence corr sponding to SEQ ID NO:9 (Sutton et al. 1991 Mol. Cell. Biol. 11: 2133-2148);

Mouse GRP78 having a polypeptide sequence corresponding to SEQ ID NO:10;

Hamster GRP78 having a polypeptide sequence corresponding to SEQ ID NO:11;

Human GRP78 having a nucleotide sequence corresponding to SEQ ID NO:12 (Ting et al. 1988 DNA 7: 275-286);

Mouse HSC70 having a nucleotide sequence corresponding to SEQ ID NO:13 and a polypeptide sequence corresponding to SEQ ID NO:14 (Giebel et al. 1988 Dev. Biol. 125: 200-207);

Human HSC70 having a nucleotide sequence corresponding to SEQ ID NO:15 (Dworniczak et al. 1987 Nucleic Acids Res. 15: 5181-5197);

Chicken GRP78 having a polypeptide sequence corresponding to SEQ ID NO:16;

Rat GRP78 as in Chang et al. (1987 Proc. Natl. Acad. Sci. USA 84: 680-684);

Saccharomyces cerevisiae SCC-1 as in Craig et al. (1987 Proc. Natl. Acad. Sci. USA 84: 680-684);

Preferred hsp70 proteins of the present invention are normally present in the endoplasmic reticulum of the cell. Preferred hsp70 proteins also include yeast KAR2, BiP, and HSP70 proteins, avian BiP or GRP78 proteins and mammalian BiP or GRP78 proteins.

The polypeptide sequence for the present PDI chaperones preferably has at least 50% homology with the yeast PDI of SEQ ID NO:18 or the rat PDI of SEQ ID NO:20. Preferred PDI chaperone polypeptides include, for example,

Saccharomyces cerevisiae PDI having a nucleotide sequence corresponding to SEQ ID NO:17 and a polypeptide sequence corresponding to SEQ ID NO:18 (La Mantia et al., 1991, Proc. Natl. Acad. Sci. USA 88: 4453-4457).

Rat PDI having a nucleotide sequence corresponding to SEQ ID NO:19 and a polypeptide sequence corresponding to SEQ ID NO:20 (Edman et al., 1985 Nature, 317:267).

Human prolyl 4-hydroxylase ß-subunit having a nucleotide and amino acid sequence as disclosed by Pihlajaniemi et al., 1987, EMBO, J. 6: 643-649.

Bovine T3BP having a nucleotide and amino acid sequence as disclosed by Yamauchi et al, 1987, Biochem. Biophys. Res. Commun., 146:1485-1492.

Murine ERp59 having a nucleotide and amino acid sequence as disclosed by Mazzarella et al., 1990, J. Biol. Chem. 265: 1094-1101.

As is known to the skilled artisan, a given amino acid is encoded by different three-nucleotide codons. Such degeneracy in the genetic code therefore means that the same polypeptide sequence can be encoded by numerous nucleotide sequences. The present invention is directed to methods utilizing any nucleotide sequence which can encode the present hsp70 chaperone polypeptides. Therefore, for example, while the KAR2 polypeptide sequence of SEQ ID NO:2 can be encoded by a nucleic acid comprising SEQ ID NO:1 there are alternative nucleic acid sequences which can encode the same KAR2 SEQ ID NO:2 polypeptide sequence. The present invention is also directed to use of such alternative nucleic acid sequences in the present methods.

Moreover when the host cell is a yeast host cell the chaperone protein is preferably a yeast KAR2 or

BiP protein or PDI protein, e.g. SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:18 and homologues thereof. Accordingly the present invention also provides a method for increasing secretion of an overexpressed gene product present in or provided to a yeast host cell, which includes expressing at least one KAR2 or BiP or PDI chaperone protein in the host cell and thereby increasing secretion of the gene product. In one embodiment such a method can also include expressing at least one of a KAR2 or BiP or PDI chaperone protein encoded by at least one expression vector present in or provided to the host cell, and thereby increasing secretion of the overexpressed recombinant gene product. Such an expression vector can include a nucleic acid encoding a polypeptide sequence for a yeast KAR2 or BiP or PDI chaperone protein operably linked to a nucleic acid which effects expression of the yeast KAR2 or BiP or PDI chaperone protein.

Yeast as used herein includes such species as Saccharomyces cerevisiae, Hansenula polymorpha, Kluyveromyces lactis, Pichia pastoris, Schizosaccharomyces pombe, Yarrowia lipolytica and the like.

Furthermore, when an avian or mammalian host is used a BiP or GRP78 or mammalian PDI chaperone protein is preferably employed, e.g. any one of SEQ ID NO: 10-12, 16 or 20 and homologues thereof. Therefore, the present invention also provides a method for increasing secretion of an overexpressed gene product in a mammalian host cell, which includes expressing at least one of a BiP or GRP78 or mammalian PDI chaperone protein in the host cell and thereby increasing secretion of the gene product. Such a method can also include expressing a BiP or GRP78 or mammalian PDI

chaperone protein encoded by an expression vector pr sent in or provided to the host cell and thereby increasing the secretion of the overexpressed gene product. Such an expression vector can include a nucleic acid encoding a polypeptide sequence for the BiP or the GRP78 or the mammalian PDI chaperone protein operably linked to a sequence which effects expression of such a chaperone protein.

In a preferred embodiment the chaperone protein is a mammalian or avian GRP78 protein, or a mammalian PDI.

Mammals as used herein includes mouse, hamster, rat, monkey, human and the like.

The present invention provides methods for increasing secretion of any overexpressed gene product which naturally has a secretion signal or has been genetically engineered to have a secretion signal.

Secretion signals are discrete amino acid sequences which cause the host cell to direct a gene product through internal and external cellular membranes and into the extracellular environment.

Secretion signals are present at the N-terminus of a nascent polypeptide gene product targeted for secretion. Additional eukaryotic secretion signals can also be present along the polypeptide chain of the gene product in the form of carbohydrates attached to specific amino acids, i.e. glycosylation secretion signals.

N-terminal signal sequences include a hydrophobic domain of about 10 to about 30 amino acids which can be preceded by a short charged domain of about 2 to about 10 amino acids. Moreover, the signal sequence is present at the N-terminus of gene products destined for secretion. In general, the particular

sequence of a signal sequence is not critical but signal sequences are rich in hydrophobic amino acids such as alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), phenylalanine (Phe), tryptophan (Trp), methionine (Met) and the like.

Many signal sequences are known (Michaelis et al. 1982 Ann. Rev. Microbiol. 36: 425). For example, the yeast acid phosphatase, yeast invertase and the yeast α-factor signal sequences have been attached to heterologous polypeptide coding regions and used successfully for secretion of the heterologous polypeptide (Sato et al. 1989 Gene 83: 355-365; Chang et al. 1986 Mol. Cell. Biol. 6: 1812-1819; and Brake et al. 1984 Proc. Natl. Acad. Sci. USA 81: 4642-4646). Therefore, the skilled artisan can readily design or obtain a nucleic acid which encodes a coding region for an overexpressed gene product which also has a signal sequence at the 5'-end.

Eukaryotic glycosylation signals include specific types of carbohydrates which are attached to specific types of amino acids present in a gene product. Carbohydrates which are attached to such amino acids include straight or branched chains containing glucose, fucose, mannose, galactose, N-acetylglucosamine, N-acetylgalactosamine, N-acetylneuraminic acid and the like. Amino acids which are frequently glycosylated include asparagine (Asn), serine (Ser), threonine (Thr), hydroxylysine and the like.

Examples of overexpressed gene products which are preferably secreted by the present methods include mammalian gene products such as enzymes, cytokines, growth factors, hormones, vaccines, antibodies and the like. More particularly, preferred overexpressed gene products of the present invention include gene products

such as erythropoietin, insulin, somatotropin, growth hormone releasing factor, platelet derived growth factor, epidermal growth factor, transforming growth factor α, transforming growth factor ß, epidermal growth factor, fibroblast growth factor, nerve growth factor, insulin-like growth factor I, insulin-like growth factor II, clotting Factor VIII, superoxide dismutase, α-interferon, γ-interferon, interleukin-1, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, granulocyte colony stimulating factor, multi-lineage colony stimulating activity, granulocyte-macrophage stimulating factor, macrophage colony stimulating factor, T cell growth factor, lymphotoxin and the like. Preferred overexpressed gene products are human gene products.

Moreover, the present methods can readily be adapted to enhance secretion of any overexpressed gene product which can be used as a vaccine. Overexpressed gene products which can be used as vaccines include any structural, membrane-associated, membrane-bound or secreted gene product of a mammalian pathogen. Mammalian pathogens include viruses, bacteria, single-celled or multi-celled parasites which can infect or attack a mammal. For example, viral vaccines can include vaccines against viruses such as human immunodeficiency virus (HIV), R. rickettsii, vaccinia, Shigella, poliovirus, adenovirus, influenza, hepatitis A, hepatitis B, dengue virus, Japanese B encephalitis, Varicella zoster, cytomegalovirus, hepatitis A, rotavirus, as well as vaccines against viral diseases like Lyme disease, measles, yellow fever, mumps, rabies, herpes, influenza, parainfluenza and the like. Bacterial vaccines can include vaccines against bacteria such as Vibrio cholerae, Salmonella typhi, Bordetella

pertussis, Streptococcus pneumoniae, Hemophilus influenza, Clostridium tetani, Corynebacterium diphtheriae, Mycobacterium leprae, Neisseria gonorrhoeae, Neisseria meningitidis, Coccidioides immitis and the like.

Moreover, an overexpressed gene product of the present invention can be overexpressed from its own natural promoter, from a mutated form of such a natural promoter or from a heterologous promoter which has been operably linked to a nucleic acid encoding the gene product. Accordingly, overexpressed gene products contemplated by the present invention include recombinant and non-recombinant gene products. As used herein a recombinant gene product is a gene product expressed from a nucleic acid which has been isolated from the natural source of such a gene product or nucleic acid. In contrast, non-recombinant, or native, gene products are expressed from nucleic acids naturally present in the host cell.

Therefore, the present overexpressed gene products can be native products of the host cell which are naturally produced at high levels, e.g. antibodies, enzymes, cytokines, hormones and the like. Moreover, if the factors controlling expression of a native gene product are understood, such factors can also be manipulated to achieve overexpression of the gene product, e.g. by induction of transcription from the natural promoter using known inducer molecules, by mutation of the nucleic acids controlling or repressing expression of the gene product to produce a mutant strain that constitutively overexpresses the gene product, by second site mutations which depress the synthesis or function of factors which normally repress the transcription of the gene product, and the like.

Similarly, the present chaperone proteins can be expressed non-recombinantly, i.e. from the host cell's native gene for that chaperone protein, by manipulating the factors controlling expression of the native chaperone protein to permit increased expression of the chaperone protein. For example, the native hsp70 chaperone gene or the transcriptional or translational control elements for the hsp70 chaperone can be mutated so that the hsp70 chaperone protein is constitutively expressed. Alternatively, nucleic acids encoding factors which control the transcription or translation of the chaperone protein can be mutated to achieve increased expression of the chaperone protein. Such mutations can thereby overcome the decrease in native chaperone protein expression which occurs upon overexpression of a gene product.

The overexpressed gene products and the chaperone proteins of the present invention can also be expressed recombinantly, i.e. by placing a nucleic acid encoding a gene product or a chaperone protein into an expression vector. Such an expression vector minimally contains a sequence which effects expression of the gene product or the chaperone protein when the sequence is operably linked to a nucleic acid encoding the gene product or the chaperone protein. Such an expression vector can also contain additional elements like origins of replication, selectable markers, transcription or termination signals, centromeres, autonomous replication sequences, and the like.

According to the present invention, first and second nucleic acids encoding an overexpressed gene product and a chaperone protein, respectively, can be placed within expression vectors to permit regulated

expression of the overexpressed gene product and/or the chaperone protein. While the chaperone protein and the overexpressed gene product can be encoded in the same expression vector, the chaperone protein is preferably encoded in an expression vector which is separate from the vector encoding the overexpressed gene product. Placement of nucleic acids encoding the chaperone protein and the overexpressed gene product in separate expression vectors can increase the amount of secreted overexpressed gene product.

As used herein, an expression vector can be a replicable or a non-replicable expression vector. A replicable expression vector can replicate either independently of host cell chromosomal DNA or because such a vector has integrated into host cell chromosomal DNA. Upon integration into host cell chromosomal DNA such an expression vector can lose some structural elements but retains the nucleic acid encoding the gene product or the hsp70 chaperone protein and a segment which can effect expression of the gene product or the chaperone protein. Therefore, the expression vectors of the present invention can be chromosomally integrating or chromosomally nonintegrating expression vectors.

In a preferred embodiment of the present invention, one or more chaperone proteins are overexpressed in a host cell by introduction of integrating or nonintegrating expression vectors into the host cell. Following introduction of at least one expression vector encoding at least one chaperone protein, the gene product is then overexpressed by inducing expression of an endogenous gene encoding the gene product, or by introducing into the host cell an expression vector encoding the gene product. In another preferred embodiment, cell lines are established which

constitutively or inducibly express at least one chaperone protein. An expression vector encoding the gene product to be overexpressed is introduced into such cell lines to achieve increased secretion of the overexpressed gene product.

The present expression vectors can be replicable in one host cell type, e.g., Escherichia coli, and undergo little or no replication in another host cell type, e.g., a eukaryotic host cell, so long as an expression vector permits expression of the present chaperone proteins or overexpressed gene products and thereby facilitates secretion of such gene products in a selected host cell type.

Expression vectors as described herein include DNA or RNA molecules engineered for controlled expression of a desired gene, i.e. a gene encoding the present chaperone proteins or a overexpressed gene product. Such vectors also encode nucleic acid segments which are operably linked to nucleic acids encoding the present chaperone polypeptides or the present overexpressed gene products. Operably linked in this context means that such segments can effect expression of nucleic acids encoding chaperone protein or overexpressed gene products. These nucleic acid sequences include promoters, enhancers, upstream control elements, transcription factors or repressor binding sites, termination signals and other elements which can control gene expression in the contemplated host cell. Preferably the vectors are plasmids, bacteriophages, cosmids or viruses.

Sambrook et al. 1989; Goeddel, 1990; Perbal, B. 1988, A Practical Guide to Molecular Cloning, John Wiley & Sons, Inc.; and Romanos et al. 1992, Yeast 8: 423-488, provide detailed reviews of vectors into which

a nucleic acid encoding the present chaperone polypeptide sequences or the contemplated overexpressed gene products can be inserted and expressed.

Expression vectors of the present invention function in yeast or mammalian cells. Yeast vectors can include the yeast 2μ circle and derivatives thereof, yeast plasmids encoding yeast autonomous replication sequences, yeast minichromosomes, any yeast integrating vector and the like. A comprehensive listing of many types of yeast vectors is provided in Parent et al. (1985 Yeast 1: 83-138). Mammalian vectors can include SV40 based vectors, polyoma based vectors, retrovirus based vectors, Epstein-Barr virus based vectors, papovavirus based vectors, bovine papilloma virus (BPV) vectors, vaccinia virus vectors, baculovirus vectors and the like. Muzyczka (ed. 1992 Curr. Top. Microbiol. Immunol. 158:97-129) provides a comprehensive review of eukaryotic expression vectors.

Elements or nucleic acid sequences capable of effecting expression of a gene product include promoters, enhancer elements, upstream activating sequences, transcription termination signals and polyadenylation sites. All such promoter and transcriptional regulatory elements, singly or in combination, are contemplated for use in the present expression vectors. Moreover, genetically-engineered and mutated regulatory sequences are also contemplated herein.

Promoters are DNA sequence elements for controlling gene expression. In particular, promoters specify transcription initiation sites and can include a TATA box and upstream promoter elements.

Yeast promoters are used in the present expression vectors when a yeast host cell is used. Such

yeast promoters include the GAL1, PGK, GAP, TPI, CYC1, ADH2, PHO5, CUP1, MFα1, MFα1 and related promoters. Romanos et al. (1992 Yeast 8: 423-488) provide a review of yeast promoters and expression vectors.

Higher eukaryotic promoters which are useful in the present expression vectors include promoters of viral origin, such as the baculovirus polyhedrin promoter, the vaccinia virus hemagglutinin (HA) promoter, SV40 early and late promoter, the herpes simplex thymidine kinase promoter, the Rous sarcoma virus LTR, the Moloney Leukemia Virus LTR, and the Murine Sarcoma Virus (MSV) LTR. Sambrook et al. (1989) and Goeddel (1990) review higher eukaryote promoters.

Preferred promoters of the present invention include inducible promoters, i.e. promoters which direct transcription at an increased or decreased rate upon binding of a transcription factor. Transcription factors as used herein include any factor that can bind to a regulatory or control region of a promoter an thereby affect transcription. The synthesis or the promoter binding ability of a transcription factor within the host cell can be controlled by exposing the host to an inducer or removing an inducer from the host cell medium. Accordingly to regulate expression of an inducible promoter, an inducer is added or removed from the growth medium of the host cell. Such inducers can include sugars, phosphate, alcohol, metal ions, hormones, heat, cold and the like. For example, commonly used inducers in yeast are glucose, galactose, and the like.

The expression vectors of the present invention can also encode selectable markers. Selectable markers are genetic functions that confer an identifiable trait upon a host cell so that cells

transformed with a vector carrying the selectable marker can be distinguished from non-transformed cells. Inclusion of a selectable marker into a vector can also be used to ensure that genetic functions linked to the marker are retained in the host cell population. Such selectable markers can confer any easily identified dominant trait, e.g. drug resistance, the ability to synthesize or metabolize cellular nutrients and the like.

Yeast selectable markers include drug resistance markers and genetic functions which allow the yeast host cell to synthesize essential cellular nutrients, e.g. amino acids. Drug resistance markers which are commonly used in yeast include chloramphenicol (Cm^r), kanamycin (kan^r), methotrexate (mtx^r or $DHFR^+$) G418 (geneticin) and the like. Genetic functions which allow the yeast host cell to synthesize essential cellular nutrients are used with available yeast strains having auxotrophic mutations in the corresponding genomic function. Common yeast selectable markers provide genetic functions for synthesizing leucine (LEU2), tryptophan (TRP1), uracil (URA3), histidine (HIS3), lysine (LYS2) and the like.

Higher eukaryotic selectable markers can include genetic functions encoding an enzyme required for synthesis of a required nutrient, e.g. the thymidine kinase (tk), dihydrofolate reductase (DHFR), uridine (CAD), adenosine deaminase (ADA), asparagine synthetase (AS) and the like. The presence of some of these enzymatic functions can also be identified by exposing the host cell to a toxin which can be inactivated by the enzyme encoded by the selectable marker. Moreover drug resistance markers are available for higher eukaryotic host cells, e.g. aminoglycoside phosphotransferase (APH)

markers are frequently used to confer resistance to kanamycin, neomycin and geneticin, and hygromycin B phosphotransferase (hyg) confers resistance to hygromycin in higher eukaryotes. Some of the foregoing selectable markers can also be used to amplify linked genetic functions by slowly adding the appropriate substrate for the enzyme encoded by markers such as DHFR, CAD, ADA, AS and others.

Therefore the present expression vectors can encode selectable markers which are useful for identifying and maintaining vector-containing host cells within a cell population present in culture. In some circumstances selectable markers can also be used to amplify the copy number of the expression vector.

After inducing transcription from the present expression vectors to produce an RNA encoding an overexpressed gene product or a chaperone protein, the RNA is translated by cellular factors to produce the gene product or the chaperone protein.

In yeast and other eukaryotes, translation of a messenger RNA (mRNA) is initiated by ribosomal binding to the 5' cap of the mRNA and migration of the ribosome along the mRNA to the first AUG start codon where polypeptide synthesis can begin. Expression in yeast and mammalian cells generally does not require specific number of nucleotides between a ribosomal-binding site and an initiation codon, as is sometimes required in prokaryotic expression systems. However, for expression in a yeast or a mammalian host cell, the first AUG codon in an mRNA is preferably the desired translational start codon.

Moreover, when expression is performed in a yeast host cell the presence of long untranslated leader sequences, e.g. longer than 50-100 nucleotides, can

diminish translation of an mRNA. Yeast mRNA leader sequences have an average length of about 50 nucleotides, are rich in adenine, have little secondary structure and almost always use the first AUG for initiation (Romanos et al. 1992; and Cigan et al. 1987 Gene 59: 1-18). Since leader sequences which do not have these characteristics can decrease the efficiency of protein translation, yeast leader sequences are preferably used for expression of an overexpressed gene product or a chaperone protein in a yeast host cell. The sequences of many yeast leader sequences are known and are available to the skilled artisan, e.g. by reference to Cigan et al. (1987 Gene 59: 1-18).

In mammalian cells, nucleic acids encoding chaperone proteins or overexpressed gene products generally include the natural ribosomal-binding site and initiation codon because, while the number of nucleotides between transcription and translational start sites can vary, such variability does not greatly affect the expression of the polypeptide in a mammalian host. However, when expression is performed in a mammalian host cell, the first AUG codon in an mRNA is preferably the desired translational start codon.

In addition to the promoter, the ribosomal-binding site and the position of the start codon, factors which can effect the level of expression obtained include the copy number of a replicable expression vector. The copy number of a vector is generally determined by the vector's origin of replication and any cis-acting control elements associated therewith. For example, an increase in copy number of a yeast episomal vector encoding a regulated centromere can be achieved by inducing transcription from a promoter which is closely juxtaposed to the

centromere (Chlebowicz-Sledziewska et al. 1985 Gene 39: 25-31). Moreover, encoding the yeast FLP function in a yeast vector can also increase the copy number of the vector (Romanos et al.).

The skilled artisan has available many choices of expression vectors. For example, commonly available yeast expression vectors include pWYG-4, pWYG7L and the like. Goeddel (1990) provides a comprehensive listing of yeast expression vectors and sources for such vectors. Commercially available higher eukaryotic expression vectors include pSVL, pMSG, pKSV-10, pSVN9 and the like.

One skilled in the art can also readily design and make expression vectors which include the above-described sequences by combining DNA fragments from available vectors, by synthesizing nucleic acids encoding such regulatory elements or by cloning and placing new regulatory elements into the present vectors. Methods for making expression vectors are well-known. Overexpressed DNA methods are found in any of the myriad of standard laboratory manuals on genetic engineering (Sambrook et al., 1989; Goeddel, 1990 and Romanos et al. 1992).

For example, a centromere-containing YCp50 vector (Goeddel, 1990) which encodes a URA3 selectable marker can be modified to encode an associated inverted sequence which permits high copy number replication in yeast. A galactose inducible promoter, e.g. PGAL1, can be placed within such a vector and a chaperone polypeptide sequence, e.g., SEQ ID NO:2 can be inserted immediately downstream. A pSC101 origin of replication can also be used in such a vector to permit replication at low copy numbers in Escherichia coli. One such replicable expression vector which has such structural

elements is a pMR1341 vector (Vogel et al. 1990 J. Cell. Biol. 110: 1885).

The expression vectors of the present invention can be made by ligating the present chaperone protein coding regions in the proper orientation to the promoter and other sequence elements being used to control gene expression. This juxtapositioning of promoter and other sequence elements with the present hsp70 chaperone polypeptide coding regions allows synthesis of large amounts of the chaperone polypeptide which can then increase secretion of a co-synthesized overexpressed protein.

After construction of the present expression vectors, such vectors are transformed into host cells where the overexpressed gene product and the chaperone protein can be expressed. Methods for transforming yeast and higher eukaryotic cells with expression vectors are well known and readily available to the skilled artisan.

For example, expression vectors can be transformed into yeast cells by any of several procedures including lithium acetate, spheroplast, electroporation and similar procedures. Such procedures can be found in numerous references including Ito et al. (1983, J. Bacteriol. 153: 163), Hinnen et al. (1978 Proc. Natl. Acad. Sci. U.S.A. 75: 1929) and Guthrie et al. (1991 Guide to Yeast Genetics and Molecular Biology, in Methods In Enzymology, vol. 194, Academic Press, New York).

Mammalian host cells can also be transformed with the present expression vectors by a variety of techniques including transfection, infection and other transformation procedures. For example, transformation procedures include calcium phosphate-mediated, DEAE-

dextran-mediated or polybrene-mediated transformation, protoplast or liposomal fusion, electroporation, direct microinjection into nuclei and the like. Such procedures are provided in Sambrook et al. and the references cited therein.

Yeast host cells which can be used with yeast replicable expression vectors include any wild type or mutant strain of yeast which is capable of secretion. Such strains can be derived from Saccharomyces cerevisiae, Hansenula polymorpha, Kluyveromyces lactis, Pichia pastoris, Schizosaccharomyces pombe, Yarrowia lipolytica and related species of yeast. In general, preferred mutant strains of yeast are strains which have a genetic deficiency that can be used in combination with a yeast vector encoding a selectable marker. Many types of yeast strains are available from the Yeast Genetics Stock Center (Donner Laboratory, University of California, Berkeley, CA 94720), the American Type Culture Collection (12301 Parklawn Drive, Rockville, MD 20852, hereinafter ATCC), the National Collection of Yeast Cultures (Food Research Institute, Colney Lane, Norwich NR4 7UA, UK) and the Centraalbureau voor Schimmelcultures (Yeast Division, Julianalaan 67a, 2628 BC Delft, Netherlands).

Tissue culture cells that are used with eukaryotic expression vectors can include VERO cells, MRC-5 cells, SCV-1 cells, COS-1 cells, CV-1 cells, LCC-MK_2 cells, NIH3T3 cells, CHO-K1 cells, mouse L cells, HeLa cells, Antheraea eucalypti moth ovarian cells, Aedes aegypti mosquito cells, S. frugiperda cells and other cultured cell lines known to one skilled in the art. Such host cells can be obtained from the ATCC. For example, Table 1 provides examples of higher eukaryotic host cells which are illustrative of the many

types of host cells which can be used with the present methods. The subject matter of Table 1 is not intended to limit the invention is any respect.

The following Examples further illustrate the invention.

TABLE 1

HOST CELL	ORIGIN	SOURCE
Aedes aegypti	Mosquito Larvae	*ATCC #CCL 125
LtK-	Mouse	Exp. Cell. Res 31:297-312
CV-1	African Green Monkey Kidney	ATCC #CCL 70
LCC-MK_2 original	Rhesus Monkey Kidney	ATCC #CCL 7
LCC-MK_2 derivative	Rhesus Monkey Kidney	ATCC #CCL 7.1
3T3	Mouse Embryo Fibroblasts	ATCC #CCL 92
CHO-K1	Chinese Hamster Ovary	ATCC #CCL 61
293	Human Embryonic Kidney	ATCC #CRL 1573
Antheraea eucalypti	Moth Ovarian Tissue	ATCC #CCL 80
HeLa	Human Cervix Epitheloid	ATCC #CCL 2
C1271	Mouse Fibroblast	ATCC #CRL 1616
HS-Sultan	Human Plasma Cell Plasmacytoma	ATCC #CRL 1484
Saccharomyces cerevisiae DBY746		ATCC #44773

* American Type Culture Collection, 1201 Parklawn Drive, Rockville, Maryland

EXAMPLE 1

EFFECT OF OVEREXPRESSION OF PROTEINS ON NATIVE YEAST CHAPERONE PROTEIN SYNTHESIS

The expression of native yeast chaperone KAR2 protein was observed in yeast cells constitutively overexpressing human gene products erythropoietin, granulocyte colony stimulating factor, platelet derived growth factor or Schizosaccharomyces pombe acid phosphatase. These non-yeast products have a variety of distinct structural features including different sizes, differences in glycosylation, and different numbers of subunits (Table 2).

TABLE 2: STRUCTURAL FEATURES OF OVEREXPRESSED GENE PRODUCTS

Protein[a]	Multiple Subunits?	Glycosylated?	Size (kd)
EPO		+	193
PDGF	+		241
GCSF			207
PHO	+	+	435
GCSF-PHO	+	+	548

[a] EPO = human erythropoietin, PDGF = human platelet derived growth factor B chain, GCSF = human granulocyte colony stimulating factor, PHO = Schizosaccharomyces pombe acid phosphatase, and GCSF-PHO = fusion between GCSF and PHO.

Materials and Methods:

Yeast YPH500 (α ura3-52 lys2-801a ade2-101 trp-Δ63 his3-Δ200 leu2-Δ1) cells were transformed with multicopy plasmids encoding one of the overexpressed gene products described in Table 2, using methods provided in Guthrie et al. and then cultured in protein-free Synthetic Complete (SC) media. Extracts from 10 ml cultures of mid-exponential growing cells were prepared by glass bead disruption (Guthrie et al). Serial dilutions were made of protein extracts from strains expressing the different gene products. Equal amounts

of total protein were loaded onto a BioRad slot blotting apparatus and blots were prepared.

The blots were probed with anti-KAR2 antibody followed by goat anti-rabbit secondary antibody conjugated to alkaline phosphatase. Alkaline phosphatase enzymatic activity was detected by use of a Lumi-Phos 530^{R} substrate (Boehringer Mannheim) to form a chemi-luminescent product. Quantitation of the amount of KAR2 protein expressed in different cell extracts was by densitometric scanning of X-ray films exposed to blots treated with Lumi-Phos 530^{R}.

Results:

Fig. 1 depicts the amounts of KAR2 protein in wild type yeast and yeast strains which had been overexpressing human erythropoietin (EPO), human platelet derived growth factor B chain (PDGF), human granulocyte colony stimulating factor (GCSF), Schizosaccharomyces pombe acid phosphatase (PHO) and a fusion between GCSF and PHO (GCSF-PHO) for 50 or more generations.

Surprisingly, native soluble KAR2 protein levels were at least five-fold lower in cells expressing these foreign genes from multicopy plasmids. Lower levels of expression from a single-copy control plasmid (i.e. single-copy PHO) did not greatly diminish KAR2 protein expression.

Similar results were obtained when using a BJ5464 yeast strain (α ura3-52 trp1 leu2Δ1 his3Δ200 pep4::HIS3 prb1Δ1.6R can1 GAL), which is deficient in vacuolar proteases. Therefore, the differences in KAR2 expression were not due to differences in the levels of vacuolar proteases. Moreover, the addition of other protease inhibitors to the cell extracts did not change the relative amount of KAR2 protein observed. Further,

mixing experiments of cellular extracts containing and not containing KAR2, confirmed that proteolysis during sample preparation was negligible. Therefore, strain-dependent differences in proteolysis could not account for the observed dimunition of KAR2 protein expression in yeast strains overexpressing proteins from multicopy plasmids.

Accordingly, the amount of native KAR2 protein in cells expressing high levels of a gene product is diminished at least 5-fold.

EXAMPLE 2

CONSTRUCTION OF AN INDUCIBLE KAR2 EXPRESSION VECTOR

A pMR1341 expression vector was made from a pMR568 plasmid which encoded the yeast KAR2 chaperone protein having -55 base pairs (bp) from the ATG start codon (i.e. position 240 of SEQ ID NO: 1) to the terminus of the coding region at bp as provided in SEQ ID NO:1. The PGAL1 promoter encoded within a SalI-AatII fragment from pB622 was placed into SalI-AatII sites within pMR568 to provide a galactose inducible promoter for the KAR2 coding region. Moreover, pMR1341 encodes a URA3 selectable marker which permits selection for this vector in ura deficient yeast host cells. In later experiments the URA3 encoding nucleic acid fragment was deleted and replaced with a fragment encoding both HIS and LEU yeast selectable markers.

Fig. 2 depicts this pMR1341 expression vector for KAR2. As depicted, this vector encodes a pSC101 origin of replication (ori pSC101) and an ampicillin resistance (Amp^R) which permit replication and selection of pMR1341 in Escherichia coli. pMR1341 further encodes a yeast centromeric (CEN4) sequence and a yeast autonomous replication sequence-1 (ARS1) which permit autonomous replication in yeast host cells. Vogel et al. (1990) describe this vector in greater detail.

EXAMPLE 3

INCREASED SECRETION OF OVEREXPRESSED PROTEINS UPON EXPRESSION OF A CHAPERONE PROTEIN

The KAR2 yeast chaperone coding region was placed under the control of a galactose inducible promoter and the plasmid encoding this chimeric gene was transformed into BJ5464 yeast cells which also carried a plasmid encoding erythropoietin (EPO) under a galactose inducible promoter. These BJ5464 cells were then grown overnight in protein-free glucose medium in the absence of galactose. Expression of KAR2 and EPO proteins was induced by transfer of the BJ5464 cells into a galactose medium (SC GAL).

Cell growth after induction was monitored by observing the optical absorption of the culture at 600 nm. Cell and supernatant samples were taken at 24, 48 and 72 hours after induction. Cell samples were used for determination of KAR2 protein levels using the slot blot procedure described in Example 1. Supernatant samples were tested for the amount of secreted EPO by using the slot blot procedure with a SY14 monoclonal antibody which is specific for EPO.

Fig. 3 depicts the KAR2 expression observed in cell extracts collected at 24, 48 and 72 hours after induction. The KAR2 immunoassay values provided in Fig. 3 represent a ratio of the amount of KAR2 detected in a given yeast cell type relative to wild type yeast. KAR2 expression in wild type cells (•), cells transformed with the EPO-encoding plasmid only (•, GalEpo) and cells transformed with both the EPO-encoding plasmid and the KAR2-encoding plasmid (▲, GalEpo+GalKar2), is depicted. After induction, expression of KAR2 is initially higher in cells with the EPO-encoding plasmid than in wild type

yeast cells. However, GalEpo cellular expression of KAR2 drops to almost wild type levels by 48 hours after induction. If KAR2 expression were monitored for longer periods of time, the amount of KAR2 in the GalEPO cells would be less than wild type, as shown in Fig. 1. However, KAR2 expression at 24 hr is significantly greater in GalEpo+GalKAR2 cells which have the KAR2-encoding plasmid despite the presence of overexpressed EPO. Moreover, by 48 to 72 hours after induction, KAR2 expression is at least 4- to 5-fold higher in cells expressing additional amounts of KAR2 recombinantly than in cells expressing KAR2 from a native, genomic locus. Therefore, KAR2 expression can be boosted significantly by recombinant expression.

Fig. 4 depicts the growth of wild type cells (□), cells transformed with the EPO-encoding plasmid only (O, GalEpo) and cells transformed with both the EPO-encoding plasmid and the KAR2-encoding plasmid (Δ, GalEpo+GalKar2) after induction of EPO and KAR2 expression.

The inset provided in Fig. 4 depicts the amount of EPO secreted into the medium of cells which have the EPO-encoding plasmid only (GalEpo) compared with the amount of secreted EPO from cells having both the EPO-encoding plasmid and the KAR2-encoding plasmid (GalEpo+GalKar2). The supernatants tested were collected during exponential growth of these yeast strains at the indicated time point (arrow). As shown in the Fig. 4 inset, the amount of EPO secreted upon induction of KAR2 expression is almost five-fold higher than when no additional KAR2 chaperone protein is present.

Therefore, increasing KAR2 expression causes a substantial increase in protein secretion.

EXAMPLE 4

CONSTRUCTION OF STRAINS OVEREXPRESSING BiP AND PDI

Yeast strains were constructed which overexpress yeast BiP, PDI or both BiP and PDI.

The overexpression system for BiP utilizes the glyceraldehyde-3-phosphate dehydrogenase (GPD) constitutive promoter. A SalI-AatII fragment containing the GPD promoter was ligated into the AatII-SalI site of the pMRI341 expression vector described in Example 2, replacing the galactose (GAL1) promoter used for inducible expression of yeast BiP. A single-copy centromere plasmid containing this construct was named pGPDKAR2. BJ5464 cells were transformed with pGPDKAR2.

To construct a yeast strain that overexpresses yeast PDI, an expression cassette containing the yeast PDI gene downstream of the constitutive ADHII promoter was integrated into the chromosomal copy of PDI using LEU2 as a selective marker. Yeast strain BJ5464 with this integrated PDI expression cassette was renamed YVH10 (PDI::ADHII-PDI-Leu2 ura3-52 trp 1 leu2Δ1 his 3Δ200 pep4::H153 prb 1Δ1.6p can 1 GAL).

YVH10 cells were transformed with pGPDKAR2 to provide cells overexpressing both BiP and PDI.

Cells extracts from mid-exponential phase cultures of BJ5464, BJ5464 transformed with pGPDKAR2, YVH10, and YVH10 transformed with pGPDKAR2 were prepared. Yeast BiP and PDI were detected by chemiluminescence using α-Kar2lgG and α-PDIlgG, respectively. Densitometry was performed with an Apple Optical Scanner and analyzed with the program Image (NIH). Quantitation of band intensity was determined from three dilutions of protein and multiple time

exposures of the bands within the linear range of the film.

As demonstrated in Table 3, BiP was overexpressed approximately 5-6 fold, and PDI was overexpressed approximately 11-16 fold.

TABLE 3

	BJ5464	BJ5464 +pGPDKAR2	YVH10	YVH10 +GPDKAR2
BiP overexpressed	-	+	-	+
PDI overexpressed	-	-	+	+
Densitometry scan, αBiP	1	5.9	1.3	5.5
Densitometry scan, αPDI	1.3	1	16	11

EXAMPLE 5

INCREASED SECRETION OF OVEREXPRESSED PROTEINS UPON EXPRESSION OF A CHAPERONE PROTEIN

The four yeast strains described in Example 4 (BJ5464, BJ5464 + pGPDKAR2, YVH10, and YVH10 + pGPDKAR2) are grown for several generations in synthetic complete (S.C.) media to provide strains which overexpress neither BiP nor PDI, BiP alone, PDI alone, or both BiP and PDI, respectively. The strains are each transformed with an expression vector which directs the constitutive expression of a gene product. Supernatant samples are collected during exponential growth of the transformed cells and assayed for the presence of the secreted gene product.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Research Corporation Technologies, Inc.
101 North Wilmot Road, Suite 600
Tucson, AZ 85711-3335
(602) 748-4400

(ii) TITLE OF INVENTION: METHODS FOR INCREASING SECRETION OF RECOMBINANTLY EXPRESSED PROTEINS

(iii) NUMBER OF SEQUENCES: 20

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: SCULLY, SCOTT, MURPHY & PRESSER
(B) STREET: 400 Garden City Plaza
(C) CITY: Garden City
(D) STATE: NY
(E) COUNTRY: USA
(F) ZIP: 11530

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Scott, Anthony C.
(B) REGISTRATION NUMBER: 25,439
(C) REFERENCE/DOCKET NUMBER: 8646Z

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2780 base pairs
(B) TYPE: nucleic acid

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 285..2333

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CTCGAGCAAA GTGTAGATCC CATTAGGACT CATCATTCAT CTAATTTTGC TATGTTAGCT      60

GCAACTTTCT ATTTTAATAG AACCTTCTGG AAATTTCACC CGGCGCGGCA CCCGAGGAAC     120

TGGACAGCGT GTCGAAAAAG TTGCTTTTTT ATATAAAGGA CACGAAAAGG GTTCTCTGGA     180

AGATATAAAT ATGGCTATGT AATTCTAAAG ATTAACGTGT TACTGTTTTA CTTTTTTAAA     240

GTCCCCAAGA GTAGTCTCAA GGGAAAAAGC GTATCAAACA TACC ATG TTT TTC AAC      296
                                                 Met Phe Phe Asn
                                                   1

AGA CTA AGC GCT GGC AAG CTG CTG GTA CCA CTC TCC GTG GTC CTG TAC      344
Arg Leu Ser Ala Gly Lys Leu Leu Val Pro Leu Ser Val Val Leu Tyr
  5                  10                  15                  20

GCC CTT TTC GTG GTA ATA TTA CCT TTA CAG AAT TCT TTC CAC TCC TCC      392
Ala Leu Phe Val Val Ile Leu Pro Leu Gln Asn Ser Phe His Ser Ser
                 25                  30                  35

AAT GTT TTA GTT AGA GGT GCC GAT GAT GTA GAA AAC TAC GGA ACT GTT      440
Asn Val Leu Val Arg Gly Ala Asp Asp Val Glu Asn Tyr Gly Thr Val
            40                  45                  50

ATC GGT ATT GAC TTA GGT ACT ACT TAT TCC TGT GTT GCT GTG ATG AAA      488
Ile Gly Ile Asp Leu Gly Thr Thr Tyr Ser Cys Val Ala Val Met Lys
        55                  60                  65

AAT GGT AAG ACT GAA ATT CTT GCT AAT GAG CAA GGT AAC AGA ATC ACC      536
Asn Gly Lys Thr Glu Ile Leu Ala Asn Glu Gln Gly Asn Arg Ile Thr
    70                  75                  80

CCA TCT TAC GTG GCA TTC ACC GAT GAT GAA AGA TTG ATT GGT GAT GCT      584
Pro Ser Tyr Val Ala Phe Thr Asp Asp Glu Arg Leu Ile Gly Asp Ala
 85                  90                  95                 100

GCA AAG AAC CAA GTT GCT GCC AAT CCT CAA AAC ACC ATC TTC GAC ATT      632
Ala Lys Asn Gln Val Ala Ala Asn Pro Gln Asn Thr Ile Phe Asp Ile
                105                 110                 115
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AAG AGA TTG ATC GGT TTG AAA TAT AAC GAC AGA TCT GTT CAG AAG GAT      680
Lys Arg Leu Ile Gly Leu Lys Tyr Asn Asp Arg Ser Val Gln Lys Asp
            120                 125                 130

ATC AAG CAC TTG CCA TTT AAT GTG GTT AAT AAA GAT GGG AAG CCC GCT      728
Ile Lys His Leu Pro Phe Asn Val Val Asn Lys Asp Gly Lys Pro Ala
        135                 140                 145

GTA GAA GTA AGT GTC AAA GGA GAA AAG AAG GTT TTT ACT CCA GAA GAA      776
Val Glu Val Ser Val Lys Gly Glu Lys Lys Val Phe Thr Pro Glu Glu
    150                 155                 160

ATT TCT GGT ATG ATC TTG GGT AAG ATG AAA CAA ATT GCC GAA GAT TAT      824
Ile Ser Gly Met Ile Leu Gly Lys Met Lys Gln Ile Ala Glu Asp Tyr
165                 170                 175                 180

TTA GGC ACT AAG GTT ACC CAT GCT GTC GTT ACT GTT CCT GCT TAT TTC      872
Leu Gly Thr Lys Val Thr His Ala Val Val Thr Val Pro Ala Tyr Phe
                185                 190                 195

AAT GAC GCG CAA AGA CAA GCC ACC AAG GAT GCT GGT ACC ATC GCT GGT      920
Asn Asp Ala Gln Arg Gln Ala Thr Lys Asp Ala Gly Thr Ile Ala Gly
            200                 205                 210

TTG AAC GTT TTG AGA ATT GTT AAT GAA CCA ACC GCA GCC GCC ATT GCC      968
Leu Asn Val Leu Arg Ile Val Asn Glu Pro Thr Ala Ala Ala Ile Ala
        215                 220                 225

TAC GGT TTG GAT AAA TCT GAT AAG GAA CAT CAA ATT ATT GTT TAT GAT     1016
Tyr Gly Leu Asp Lys Ser Asp Lys Glu His Gln Ile Ile Val Tyr Asp
    230                 235                 240

TTG GGT GGT GGT ACT TTC GAT GTC TCT CTA TTG TCT ATT GAA AAC GGT     1064
Leu Gly Gly Gly Thr Phe Asp Val Ser Leu Leu Ser Ile Glu Asn Gly
245                 250                 255                 260

GTT TTC GAA GTC CAA GCC ACT TCT GGT GAT ACT CAT TTA GGT GGT GAA     1112
Val Phe Glu Val Gln Ala Thr Ser Gly Asp Thr His Leu Gly Gly Glu
                265                 270                 275

GAT TTT GAC TAT AAG ATC GTT CGT CAA TTG ATA AAA GCT TTC AAG AAG     1160
Asp Phe Asp Tyr Lys Ile Val Arg Gln Leu Ile Lys Ala Phe Lys Lys
            280                 285                 290

AAG CAT GGT ATT GAT GTG TCT GAC AAC AAC AAG GCC CTA GCT AAA TTG     1208
Lys His Gly Ile Asp Val Ser Asp Asn Asn Lys Ala Leu Ala Lys Leu
        295                 300                 305

AAG AGA GAA GCT GAA AAG GCT AAA CGT GCC TTG TCC AGC CAA ATG TCC     1256
Lys Arg Glu Ala Glu Lys Ala Lys Arg Ala Leu Ser Ser Gln Met Ser
    310                 315                 320
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ACC CGT ATT GAA ATT GAC TCC TTC GTT GAT GGT ATC GAC TTA AGT GAA      1304
Thr Arg Ile Glu Ile Asp Ser Phe Val Asp Gly Ile Asp Leu Ser Glu
325                 330                 335                 340

ACC TTG ACC AGA GCT AAG TTT GAG GAA TTA AAC CTA GAT CTA TTC AAG      1352
Thr Leu Thr Arg Ala Lys Phe Glu Glu Leu Asn Leu Asp Leu Phe Lys
                345                 350                 355

AAG ACC TTG AAG CCT GTC GAG AAG GTT TTG CAA GAT TCT GGT TTG GAA      1400
Lys Thr Leu Lys Pro Val Glu Lys Val Leu Gln Asp Ser Gly Leu Glu
            360                 365                 370

AAG AAG GAT GTT GAT GAT ATC GTT TTG GTT GGT GGT TCT ACT AGA ATT      1448
Lys Lys Asp Val Asp Asp Ile Val Leu Val Gly Gly Ser Thr Arg Ile
        375                 380                 385

CCA AAG GTC CAA CAA TTG TTA GAA TCA TAC TTT GAT GGT AAG AAG GCC      1496
Pro Lys Val Gln Gln Leu Leu Glu Ser Tyr Phe Asp Gly Lys Lys Ala
    390                 395                 400

TCC AAG GGT ATT AAC CCA GAT GAA GCT GTT GCA TAC GGT GCA GCC GTT      1544
Ser Lys Gly Ile Asn Pro Asp Glu Ala Val Ala Tyr Gly Ala Ala Val
405                 410                 415                 420

CAA GCT GGT GTC TTA TCC GGT GAA GAA GGT GTC GAA GAT ATT GTT TTA      1592
Gln Ala Gly Val Leu Ser Gly Glu Glu Gly Val Glu Asp Ile Val Leu
                425                 430                 435

TTG GAT GTC AAC GCT TTG ACT CTT GGT ATT GAA ACC ACT GGT GGT GTC      1640
Leu Asp Val Asn Ala Leu Thr Leu Gly Ile Glu Thr Thr Gly Gly Val
            440                 445                 450

ATG ACT CCA TTA ATT AAG AGA AAT ACT GCT ATT CCT ACA AAG AAA TCC      1688
Met Thr Pro Leu Ile Lys Arg Asn Thr Ala Ile Pro Thr Lys Lys Ser
        455                 460                 465

CAA ATT TTC TCT ACT GCC GTT GAC AAC CAA CCA ACC GTT ATG ATC AAG      1736
Gln Ile Phe Ser Thr Ala Val Asp Asn Gln Pro Thr Val Met Ile Lys
    470                 475                 480

GTA TAC GAG GGT GAA AGA GCC ATG TCT AAG GAC AAC AAT CTA TTA GGT      1784
Val Tyr Glu Gly Glu Arg Ala Met Ser Lys Asp Asn Asn Leu Leu Gly
485                 490                 495                 500

AAG TTT GAA TTA ACC GGC ATT CCA CCA GCA CCA AGA GGT GTA CCT CAA      1832
Lys Phe Glu Leu Thr Gly Ile Pro Pro Ala Pro Arg Gly Val Pro Gln
                505                 510                 515

ATT GAA GTC ACA TTT GCA CTT GAC GCT AAT GGT ATT CTG AAG GTG TCT      1880
Ile Glu Val Thr Phe Ala Leu Asp Ala Asn Gly Ile Leu Lys Val Ser
            520                 525                 530
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GCC ACA GAT AAG GGA ACT GGT AAA TCC GAA TCT ATC ACC ATC ACT AAC      1928
Ala Thr Asp Lys Gly Thr Gly Lys Ser Glu Ser Ile Thr Ile Thr Asn
        535                 540                 545

GAT AAA GGT AGA TTA ACC CAA GAA GAG ATT GAT AGA ATG GTT GAA GAG      1976
Asp Lys Gly Arg Leu Thr Gln Glu Glu Ile Asp Arg Met Val Glu Glu
    550                 555                 560

GCT GAA AAA TTC GCT TCT GAA GAC GCT TCT ATC AAG GCC AAG GTT GAA      2024
Ala Glu Lys Phe Ala Ser Glu Asp Ala Ser Ile Lys Ala Lys Val Glu
565                 570                 575                 580

TCT AGA AAC AAA TTA GAA AAC TAC GCT CAC TCT TTG AAA AAC CAA GTT      2072
Ser Arg Asn Lys Leu Glu Asn Tyr Ala His Ser Leu Lys Asn Gln Val
                585                 590                 595

AAT GGT GAC CTA GGT GAA AAA TTG GAA GAA GAA GAC AAG GAA ACC TTA      2120
Asn Gly Asp Leu Gly Glu Lys Leu Glu Glu Glu Asp Lys Glu Thr Leu
            600                 605                 610

TTA GAT GCT GCT AAC GAT GTT TTA GAA TGG TTA GAT GAT AAC TTT GAA      2168
Leu Asp Ala Ala Asn Asp Val Leu Glu Trp Leu Asp Asp Asn Phe Glu
        615                 620                 625

ACC GCC ATT GCT GAA GAC TTT GAT GAA AAG TTC GAA TCT TTG TCC AAG      2216
Thr Ala Ile Ala Glu Asp Phe Asp Glu Lys Phe Glu Ser Leu Ser Lys
    630                 635                 640

GTC GCT TAT CCA ATT ACT TCT AAG TTG TAC GGA GGT GCT GAT GGT TCT      2264
Val Ala Tyr Pro Ile Thr Ser Lys Leu Tyr Gly Gly Ala Asp Gly Ser
645                 650                 655                 660

GGT GCC GCT GAT TAT GAC GAC GAA GAT GAA GAT GAC GAT GGT GAT TAT      2312
Gly Ala Ala Asp Tyr Asp Asp Glu Asp Glu Asp Asp Asp Gly Asp Tyr
                665                 670                 675

TTC GAA CAC GAC GAA TTG TAGATAAAAT AGTTAAAAAT TTTTGCTGCT             2360
Phe Glu His Asp Glu Leu
            680

GGAAGCTTCA AGGTTGTTAA TTTATTGACT TGCATAGAAT ATCTACATTT CTTCTAAAAA    2420

TACATGCATA GCTAATTCAA ACTTCGAGCT TCATACAATT TTCGAGGAGA TTATACTGAG    2480

TATATACGTA AATATATGCA TTATATGTTA TAAAATTAGA AAGATATAGA AATTTCATTG    2540

AAGAGTATAG AGACTGGGGT TAAGGTACTC AGTAACAGTG TCATCAATAT GCTAATTTTG    2600

CGTATTACTT AGCTCTATTG CGCAAATGCA ATTTTTTCTT ACCCTGATAA TGCTTTATTT    2660
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CCCGTTCCGA AAATTTTTCA CTGAAAAAAA AGTGCTTAAG CTCATCTCAT CTCATCTCAT 2720

CCCATCACTA TTGAAATATT TTGCTAAAAC ATTATAACAG AGAGAGTTGA AAGGCTCGAG 2780

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 682 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Phe Phe Asn Arg Leu Ser Ala Gly Lys Leu Leu Val Pro Leu Ser
 1               5                  10                  15

Val Val Leu Tyr Ala Leu Phe Val Val Ile Leu Pro Leu Gln Asn Ser
            20                  25                  30

Phe His Ser Ser Asn Val Leu Val Arg Gly Ala Asp Asp Val Glu Asn
        35                  40                  45

Tyr Gly Thr Val Ile Gly Ile Asp Leu Gly Thr Thr Tyr Ser Cys Val
    50                  55                  60

Ala Val Met Lys Asn Gly Lys Thr Glu Ile Leu Ala Asn Glu Gln Gly
65                  70                  75                  80

Asn Arg Ile Thr Pro Ser Tyr Val Ala Phe Thr Asp Asp Glu Arg Leu
                85                  90                  95

Ile Gly Asp Ala Ala Lys Asn Gln Val Ala Ala Asn Pro Gln Asn Thr
            100                 105                 110

Ile Phe Asp Ile Lys Arg Leu Ile Gly Leu Lys Tyr Asn Asp Arg Ser
        115                 120                 125

Val Gln Lys Asp Ile Lys His Leu Pro Phe Asn Val Val Asn Lys Asp
    130                 135                 140

Gly Lys Pro Ala Val Glu Val Ser Val Lys Gly Glu Lys Lys Val Phe
145                 150                 155                 160

Thr Pro Glu Glu Ile Ser Gly Met Ile Leu Gly Lys Met Lys Gln Ile
                165                 170                 175
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Ala Glu Asp Tyr Leu Gly Thr Lys Val Thr His Ala Val Val Thr Val
            180                 185                 190

Pro Ala Tyr Phe Asn Asp Ala Gln Arg Gln Ala Thr Lys Asp Ala Gly
        195                 200                 205

Thr Ile Ala Gly Leu Asn Val Leu Arg Ile Val Asn Glu Pro Thr Ala
    210                 215                 220

Ala Ala Ile Ala Tyr Gly Leu Asp Lys Ser Asp Lys Glu His Gln Ile
225                 230                 235                 240

Ile Val Tyr Asp Leu Gly Gly Gly Thr Phe Asp Val Ser Leu Leu Ser
                245                 250                 255

Ile Glu Asn Gly Val Phe Glu Val Gln Ala Thr Ser Gly Asp Thr His
            260                 265                 270

Leu Gly Gly Glu Asp Phe Asp Tyr Lys Ile Val Arg Gln Leu Ile Lys
        275                 280                 285

Ala Phe Lys Lys Lys His Gly Ile Asp Val Ser Asp Asn Asn Lys Ala
    290                 295                 300

Leu Ala Lys Leu Lys Arg Glu Ala Glu Lys Ala Lys Arg Ala Leu Ser
305                 310                 315                 320

Ser Gln Met Ser Thr Arg Ile Glu Ile Asp Ser Phe Val Asp Gly Ile
                325                 330                 335

Asp Leu Ser Glu Thr Leu Thr Arg Ala Lys Phe Glu Glu Leu Asn Leu
            340                 345                 350

Asp Leu Phe Lys Lys Thr Leu Lys Pro Val Glu Lys Val Leu Gln Asp
        355                 360                 365

Ser Gly Leu Glu Lys Lys Asp Val Asp Asp Ile Val Leu Val Gly Gly
    370                 375                 380

Ser Thr Arg Ile Pro Lys Val Gln Gln Leu Leu Glu Ser Tyr Phe Asp
385                 390                 395                 400

Gly Lys Lys Ala Ser Lys Gly Ile Asn Pro Asp Glu Ala Val Ala Tyr
                405                 410                 415

Gly Ala Ala Val Gln Ala Gly Val Leu Ser Gly Glu Glu Gly Val Glu
            420                 425                 430

Asp Ile Val Leu Leu Asp Val Asn Ala Leu Thr Leu Gly Ile Glu Thr
        435                 440                 445
```

Thr Gly Gly Val Met Thr Pro Leu Ile Lys Arg Asn Thr Ala Ile Pro
450 455 460

Thr Lys Lys Ser Gln Ile Phe Ser Thr Ala Val Asp Asn Gln Pro Thr
465 470 475 480

Val Met Ile Lys Val Tyr Glu Gly Glu Arg Ala Met Ser Lys Asp Asn
485 490 495

Asn Leu Leu Gly Lys Phe Glu Leu Thr Gly Ile Pro Pro Ala Pro Arg
500 505 510

Gly Val Pro Gln Ile Glu Val Thr Phe Ala Leu Asp Ala Asn Gly Ile
515 520 525

Leu Lys Val Ser Ala Thr Asp Lys Gly Thr Gly Lys Ser Glu Ser Ile
530 535 540

Thr Ile Thr Asn Asp Lys Gly Arg Leu Thr Gln Glu Glu Ile Asp Arg
545 550 555 560

Met Val Glu Glu Ala Glu Lys Phe Ala Ser Glu Asp Ala Ser Ile Lys
565 570 575

Ala Lys Val Glu Ser Arg Asn Lys Leu Glu Asn Tyr Ala His Ser Leu
580 585 590

Lys Asn Gln Val Asn Gly Asp Leu Gly Glu Lys Leu Glu Glu Glu Asp
595 600 605

Lys Glu Thr Leu Leu Asp Ala Ala Asn Asp Val Leu Glu Trp Leu Asp
610 615 620

Asp Asn Phe Glu Thr Ala Ile Ala Glu Asp Phe Asp Glu Lys Phe Glu
625 630 635 640

Ser Leu Ser Lys Val Ala Tyr Pro Ile Thr Ser Lys Leu Tyr Gly Gly
645 650 655

Ala Asp Gly Ser Gly Ala Ala Asp Tyr Asp Asp Glu Asp Glu Asp Asp
660 665 670

Asp Gly Asp Tyr Phe Glu His Asp Glu Leu
675 680

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2367 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 251..2176

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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AAGCTTTTAG GAATTTTGAA TTTTTGATCG AATTTTAGAA AAAACTATTC GCAAGACTAC      60

AATTTTTGAA GGGTGCTATT TGTGAAAAAA TAAAACGTGA AATAAATCGT TTTATAATTT     120

ACGAATTGTC GTTATTCAAA ACTCAAAAAA TATGATCTCG TCGAGATTCA CTAATGTAGT     180

CCGTAGCGGA TTGCGTTTCC AAAGCAAGGG AGCATCGTTC AAGATTGGCG CTTCCTTGCA     240

TGGAAGTCGC ATG ACC GCC CGC TGG AAT TCT AAT GCA AGT GGT AAT GAA          289
          Met Thr Ala Arg Trp Asn Ser Asn Ala Ser Gly Asn Glu
            1               5                  10

AAA GTT AAG GGT CCC GTA ATC GGT ATT GAC TTG GGT ACC ACC ACC TCA        337
Lys Val Lys Gly Pro Val Ile Gly Ile Asp Leu Gly Thr Thr Thr Ser
     15                  20                  25

TGT TTA GCA ATC ATG GAG GGT CAA ACC CCT AAG GTT ATT GCA AAT GCC        385
Cys Leu Ala Ile Met Glu Gly Gln Thr Pro Lys Val Ile Ala Asn Ala
 30                  35                  40                  45

GAG GGT ACC CGT ACC ACA CCA TCT GTC GTC GCA TTT ACC AAA GAT GGC        433
Glu Gly Thr Arg Thr Thr Pro Ser Val Val Ala Phe Thr Lys Asp Gly
                50                  55                  60

GAG CGT TTG GTG GGT GTT AGC GCT AAA CGC CAA GCC GTC ATT AAC CCG        481
Glu Arg Leu Val Gly Val Ser Ala Lys Arg Gln Ala Val Ile Asn Pro
            65                  70                  75

GAA AAC ACA TTT TTT GCT ACT AAG CGT TTA ATC GGT CGT AGA TTT AAA        529
Glu Asn Thr Phe Phe Ala Thr Lys Arg Leu Ile Gly Arg Arg Phe Lys
        80                  85                  90

GAG CCT GAA GTC CAA CGT GAT ATT AAG GAA GTT CCT TAC AAA ATT GTC        577
Glu Pro Glu Val Gln Arg Asp Ile Lys Glu Val Pro Tyr Lys Ile Val
     95                  100                 105
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GAG CAC TCA AAT GGA GAT GCT TGG TTG GAG GCT CGT GGT AAG ACC TAC      625
Glu His Ser Asn Gly Asp Ala Trp Leu Glu Ala Arg Gly Lys Thr Tyr
110                 115                 120                 125

TCT CCA TCT CAA ATC GGT GGT TTC ATC CTT AGT AAG ATG AGG GAA ACT      673
Ser Pro Ser Gln Ile Gly Gly Phe Ile Leu Ser Lys Met Arg Glu Thr
                130                 135                 140

GCC AGC ACC TAC CTT GGA AAA GAT GTA AAG AAT GCC GTT GTT ACT GTT      721
Ala Ser Thr Tyr Leu Gly Lys Asp Val Lys Asn Ala Val Val Thr Val
            145                 150                 155

CCT GCT TAC TTC AAT GAC TCT CAG CGT CAA GCT ACC AAG GCT GCT GGT      769
Pro Ala Tyr Phe Asn Asp Ser Gln Arg Gln Ala Thr Lys Ala Ala Gly
        160                 165                 170

GCC ATT GCT GGT TTG AAT GTT TTG CGT GTC GTC AAC GAG CCT ACT GCC      817
Ala Ile Ala Gly Leu Asn Val Leu Arg Val Val Asn Glu Pro Thr Ala
    175                 180                 185

GCC GCT TTG GCT TAT GGT TTG GAC AAG AAG AAT GAT GCC ATC GTC GCA      865
Ala Ala Leu Ala Tyr Gly Leu Asp Lys Lys Asn Asp Ala Ile Val Ala
190                 195                 200                 205

GTT TTC GAT TTG GGT GGT GGT ACT TTT GAT ATT TCT ATT TTG GAG TTA      913
Val Phe Asp Leu Gly Gly Gly Thr Phe Asp Ile Ser Ile Leu Glu Leu
                210                 215                 220

AAC AAT GGT GTT TTT GAG GTT AGA AGT ACC AAC GGT GAC ACT CAT TTG      961
Asn Asn Gly Val Phe Glu Val Arg Ser Thr Asn Gly Asp Thr His Leu
            225                 230                 235

GGT GGT GAG GAC TTT GAT GTT GCT CTT GTT CGT CAC ATT GTC GAG ACC     1009
Gly Gly Glu Asp Phe Asp Val Ala Leu Val Arg His Ile Val Glu Thr
        240                 245                 250

TTT AAG AAG AAT GAG GGT TTG GAC TTG AGC AAG GAC CGT CTC GCC GTT     1057
Phe Lys Lys Asn Glu Gly Leu Asp Leu Ser Lys Asp Arg Leu Ala Val
    255                 260                 265

CAA CGT ATT CGT GAG GCT GCT GAA AAA GCT AAG TGC GAA CTT TCC TCT     1105
Gln Arg Ile Arg Glu Ala Ala Glu Lys Ala Lys Cys Glu Leu Ser Ser
270                 275                 280                 285

CTT TCC AAG ACT GAT ATC AGT CTT CCT TTC ATT ACT GCG GAT GCT ACT     1153
Leu Ser Lys Thr Asp Ile Ser Leu Pro Phe Ile Thr Ala Asp Ala Thr
                290                 295                 300

GGC CCT AAG CAT ATT AAC ATG GAA ATC TCT CGT GCT CAA TTT GAG AAA     1201
Gly Pro Lys His Ile Asn Met Glu Ile Ser Arg Ala Gln Phe Glu Lys
            305                 310                 315
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CTT GTT GAT CCT CTC GTT CGT CGT ACC ATC GAT CCT TGC AAG CGT GCC      1249
Leu Val Asp Pro Leu Val Arg Arg Thr Ile Asp Pro Cys Lys Arg Ala
        320                 325                 330

CTT AAG GAT GCT AAC TTG CAA ACC TCT GAA ATC AAT GAA GTT ATC CTT      1297
Leu Lys Asp Ala Asn Leu Gln Thr Ser Glu Ile Asn Glu Val Ile Leu
    335                 340                 345

GTC GGT GGT ATG ACT CGT ATG CCT CGT GTT GTC GAA ACT GTC AAG AGT      1345
Val Gly Gly Met Thr Arg Met Pro Arg Val Val Glu Thr Val Lys Ser
350                 355                 360                 365

ATC TTC AAG CGT GAA CCC GCT AAG TCC GTC AAC CCT GAT GAA GCT GTT      1393
Ile Phe Lys Arg Glu Pro Ala Lys Ser Val Asn Pro Asp Glu Ala Val
                370                 375                 380

GCC ATT GGT GCT GCT ATT CAA GGT GGT GTC TTG TCT GGC CAT GTT AAG      1441
Ala Ile Gly Ala Ala Ile Gln Gly Gly Val Leu Ser Gly His Val Lys
            385                 390                 395

GAC CTT GTT CTT TTG GAT GTC ACC CCC TTG TCC CTC GGT ATC GAG ACT      1489
Asp Leu Val Leu Leu Asp Val Thr Pro Leu Ser Leu Gly Ile Glu Thr
        400                 405                 410

TTG GGC GGT GTT TTC ACT CGT TTG ATC AAC CGT AAC ACT ACC ATT CCT      1537
Leu Gly Gly Val Phe Thr Arg Leu Ile Asn Arg Asn Thr Thr Ile Pro
    415                 420                 425

ACT CGC AAG TCT CAA GTT TTC TCC ACT GCT GCT GAT GGT CAA ACT GCC      1585
Thr Arg Lys Ser Gln Val Phe Ser Thr Ala Ala Asp Gly Gln Thr Ala
430                 435                 440                 445

GTT GAA ATC CGT GTC TTC CAG GGT GAA CGT GAG CTT GTT CGT GAC AAC      1633
Val Glu Ile Arg Val Phe Gln Gly Glu Arg Glu Leu Val Arg Asp Asn
                450                 455                 460

AAA TTA ATT GGC AAC TTC CAA CTT ACT GGC ATT GCT CCT GCA CCT AAG      1681
Lys Leu Ile Gly Asn Phe Gln Leu Thr Gly Ile Ala Pro Ala Pro Lys
            465                 470                 475

GGT CAA CCT CAG ATT GAG GTT TCT TTT GAT GTT GAT GCC GAT GGC ATT      1729
Gly Gln Pro Gln Ile Glu Val Ser Phe Asp Val Asp Ala Asp Gly Ile
        480                 485                 490

ATC AAT GTC TCT GCC CGT GAC AAG GCT ACC AAC AAG GAT TCT TCC ATC      1777
Ile Asn Val Ser Ala Arg Asp Lys Ala Thr Asn Lys Asp Ser Ser Ile
    495                 500                 505

ACT GTT GCT GGA TCT TCC GGT TTA ACT GAT TCT GAG ATT GAG GCT ATG      1825
Thr Val Ala Gly Ser Ser Gly Leu Thr Asp Ser Glu Ile Glu Ala Met
510                 515                 520                 525
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GTT GCC GAT GCT GAG AAG TAT CGT GCC AGT GAC ATG GCT CGC AAG GAG      1873
Val Ala Asp Ala Glu Lys Tyr Arg Ala Ser Asp Met Ala Arg Lys Glu
                530                 535                 540

GCT ATT GAG AAC GGA AAC AGA GCT GAA AGC GTC TGC ACC GAT ATT GAA      1921
Ala Ile Glu Asn Gly Asn Arg Ala Glu Ser Val Cys Thr Asp Ile Glu
            545                 550                 555

AGC AAC CTT GAC ATT CAC AAA GAC AAA TTG GAC CAA CAA GCT GTT GAA      1969
Ser Asn Leu Asp Ile His Lys Asp Lys Leu Asp Gln Gln Ala Val Glu
        560                 565                 570

GAC TTG CGC TCC AAG ATC ACC GAT CTC CGT GAA ACT GTT GCC AAG GTC      2017
Asp Leu Arg Ser Lys Ile Thr Asp Leu Arg Glu Thr Val Ala Lys Val
    575                 580                 585

AAC GCT GGT GAC GAA GGT ATT ACT AGT GAA GAT ATG AAG AAG AAG ATT      2065
Asn Ala Gly Asp Glu Gly Ile Thr Ser Glu Asp Met Lys Lys Lys Ile
590                 595                 600                 605

GAT GAA ATT CAA CAA CTC TCT TTG AAG GTT TTC GAG TCT GTC TAC AAG      2113
Asp Glu Ile Gln Gln Leu Ser Leu Lys Val Phe Glu Ser Val Tyr Lys
                610                 615                 620

AAC CAA AAT CAA GGT AAT GAA TCT TCT GGT GAT AAC TCT GCT CCT GAG      2161
Asn Gln Asn Gln Gly Asn Glu Ser Ser Gly Asp Asn Ser Ala Pro Glu
            625                 630                 635

GGT GAC AAG AAG TAGAGTGCAC ACCACAGTAC GAAATGACAT GTGCAATTTT          2213
Gly Asp Lys Lys                                                     .
        640

CAATTTTAGC TCTATATGTC AAAAAATTTA TGTGGATAAT TGATTATCCA TTTACATGTT    2273

GAAAGAAAAT GTCTGGATTT TGAAAAGGTA AACTATGATA TTTTTATTAA ATGTTCTAAA    2333

AAAAAAAAAA AAAAAAAAAA AAAAACCGGA ATTC                                2367
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 641 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Thr Ala Arg Trp Asn Ser Asn Ala Ser Gly Asn Glu Lys Val Lys
1 5 10 15

Gly Pro Val Ile Gly Ile Asp Leu Gly Thr Thr Thr Ser Cys Leu Ala
20 25 30

Ile Met Glu Gly Gln Thr Pro Lys Val Ile Ala Asn Ala Glu Gly Thr
35 40 45

Arg Thr Thr Pro Ser Val Val Ala Phe Thr Lys Asp Gly Glu Arg Leu
50 55 60

Val Gly Val Ser Ala Lys Arg Gln Ala Val Ile Asn Pro Glu Asn Thr
65 70 75 80

Phe Phe Ala Thr Lys Arg Leu Ile Gly Arg Arg Phe Lys Glu Pro Glu
85 90 95

Val Gln Arg Asp Ile Lys Glu Val Pro Tyr Lys Ile Val Glu His Ser
100 105 110

Asn Gly Asp Ala Trp Leu Glu Ala Arg Gly Lys Thr Tyr Ser Pro Ser
115 120 125

Gln Ile Gly Gly Phe Ile Leu Ser Lys Met Arg Glu Thr Ala Ser Thr
130 135 140

Tyr Leu Gly Lys Asp Val Lys Asn Ala Val Val Thr Val Pro Ala Tyr
145 150 155 160

Phe Asn Asp Ser Gln Arg Gln Ala Thr Lys Ala Ala Gly Ala Ile Ala
165 170 175

Gly Leu Asn Val Leu Arg Val Val Asn Glu Pro Thr Ala Ala Ala Leu
180 185 190

Ala Tyr Gly Leu Asp Lys Lys Asn Asp Ala Ile Val Ala Val Phe Asp
195 200 205

Leu Gly Gly Gly Thr Phe Asp Ile Ser Ile Leu Glu Leu Asn Asn Gly
210 215 220

Val Phe Glu Val Arg Ser Thr Asn Gly Asp Thr His Leu Gly Gly Glu
225 230 235 240

Asp Phe Asp Val Ala Leu Val Arg His Ile Val Glu Thr Phe Lys Lys
245 250 255

```
Asn Glu Gly Leu Asp Leu Ser Lys Asp Arg Leu Ala Val Gln Arg Ile
            260                 265                 270

Arg Glu Ala Ala Glu Lys Ala Lys Cys Glu Leu Ser Ser Leu Ser Lys
        275                 280                 285

Thr Asp Ile Ser Leu Pro Phe Ile Thr Ala Asp Ala Thr Gly Pro Lys
    290                 295                 300

His Ile Asn Met Glu Ile Ser Arg Ala Gln Phe Glu Lys Leu Val Asp
305                 310                 315                 320

Pro Leu Val Arg Arg Thr Ile Asp Pro Cys Lys Arg Ala Leu Lys Asp
                325                 330                 335

Ala Asn Leu Gln Thr Ser Glu Ile Asn Glu Val Ile Leu Val Gly Gly
            340                 345                 350

Met Thr Arg Met Pro Arg Val Val Glu Thr Val Lys Ser Ile Phe Lys
        355                 360                 365

Arg Glu Pro Ala Lys Ser Val Asn Pro Asp Glu Ala Val Ala Ile Gly
    370                 375                 380

Ala Ala Ile Gln Gly Gly Val Leu Ser Gly His Val Lys Asp Leu Val
385                 390                 395                 400

Leu Leu Asp Val Thr Pro Leu Ser Leu Gly Ile Glu Thr Leu Gly Gly
                405                 410                 415

Val Phe Thr Arg Leu Ile Asn Arg Asn Thr Thr Ile Pro Thr Arg Lys
            420                 425                 430

Ser Gln Val Phe Ser Thr Ala Ala Asp Gly Gln Thr Ala Val Glu Ile
        435                 440                 445

Arg Val Phe Gln Gly Glu Arg Glu Leu Val Arg Asp Asn Lys Leu Ile
    450                 455                 460

Gly Asn Phe Gln Leu Thr Gly Ile Ala Pro Ala Pro Lys Gly Gln Pro
465                 470                 475                 480

Gln Ile Glu Val Ser Phe Asp Val Asp Ala Asp Gly Ile Ile Asn Val
                485                 490                 495

Ser Ala Arg Asp Lys Ala Thr Asn Lys Asp Ser Ser Ile Thr Val Ala
            500                 505                 510

Gly Ser Ser Gly Leu Thr Asp Ser Glu Ile Glu Ala Met Val Ala Asp
        515                 520                 525
```

```
Ala Glu Lys Tyr Arg Ala Ser Asp Met Ala Arg Lys Glu Ala Ile Glu
    530                 535                 540

Asn Gly Asn Arg Ala Glu Ser Val Cys Thr Asp Ile Glu Ser Asn Leu
545                 550                 555                 560

Asp Ile His Lys Asp Lys Leu Asp Gln Gln Ala Val Glu Asp Leu Arg
                565                 570                 575

Ser Lys Ile Thr Asp Leu Arg Glu Thr Val Ala Lys Val Asn Ala Gly
            580                 585                 590


Asp Glu Gly Ile Thr Ser Glu Asp Met Lys Lys Lys Ile Asp Glu Ile
        595                 600                 605

Gln Gln Leu Ser Leu Lys Val Phe Glu Ser Val Tyr Lys Asn Gln Asn
    610                 615                 620

Gln Gly Asn Glu Ser Ser Gly Asp Asn Ser Ala Pro Glu Gly Asp Lys
625                 630                 635                 640

Lys
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 679 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Phe Ser Ala Arg Lys Ser Ser Val Gly Trp Leu Val Ser Ser Leu
1               5                   10                  15

Ala Val Phe Tyr Val Leu Leu Ala Val Ile Met Pro Ile Ala Leu Thr
            20                  25                  30

Gly Ser Gln Ser Ser Arg Val Val Ala Arg Ala Ala Glu Asp His Glu
        35                  40                  45

Asp Tyr Gly Thr Val Ile Gly Ile Asp Leu Gly Thr Thr Tyr Ser Cys
    50                  55                  60
```

```
Val Ala Val Met Lys Asn Gly Lys Thr Glu Ile Leu Ala Asn Glu Gln
65                  70                  75                  80

Gly Asn Arg Ile Thr Pro Ser Tyr Val Ser Phe Thr Asp Asp Glu Arg
                85                  90                  95

Leu Ile Gly Asp Ala Ala Lys Asn Gln Ala Ala Ser Asn Pro Lys Asn
            100                 105                 110

Thr Ile Phe Asp Ile Lys Arg Leu Ile Gly Leu Gln Tyr Asn Asp Pro
        115                 120                 125

Thr Val Gln Arg Asp Ile Lys His Leu Pro Tyr Thr Val Val Asn Lys
    130                 135                 140

Gly Asn Lys Pro Tyr Val Glu Val Thr Val Lys Gly Glu Lys Lys Glu
145                 150                 155                 160

Phe Thr Pro Glu Glu Val Ser Gly Met Ile Leu Gly Lys Met Lys Gln
                165                 170                 175

Ile Ala Glu Asp Tyr Leu Gly Lys Lys Val Thr His Ala Val Val Thr
            180                 185                 190

Val Pro Ala Tyr Phe Asn Asp Ala Gln Arg Gln Ala Thr Lys Asp Ala
        195                 200                 205

Gly Ala Ile Ala Gly Leu Asn Ile Leu Arg Ile Val Asn Glu Pro Thr
    210                 215                 220

Ala Ala Ala Ile Ala Tyr Gly Leu Asp Lys Thr Glu Asp Glu His Gln
225                 230                 235                 240

Ile Ile Val Tyr Asp Leu Gly Gly Gly Thr Phe Asp Val Ser Leu Leu
                245                 250                 255

Ser Ile Glu Asn Gly Val Phe Glu Val Gln Ala Thr Ala Gly Asp Thr
            260                 265                 270

His Leu Gly Gly Glu Asp Phe Asp Tyr Lys Leu Val Arg His Phe Ala
        275                 280                 285

Gln Leu Phe Gln Lys Lys His Asp Leu Asp Val Thr Lys Asn Asp Lys
    290                 295                 300

Ala Met Ala Lys Leu Lys Arg Glu Ala Glu Lys Ala Lys Arg Ser Leu
305                 310                 315                 320

Ser Ser Gln Thr Ser Thr Arg Ile Glu Ile Asp Ser Phe Phe Asn Gly
                325                 330                 335
```

```
Ile Asp Phe Ser Glu Thr Leu Thr Arg Ala Lys Phe Glu Glu Leu Asn
            340                 345                 350

Leu Ala Leu Phe Lys Lys Thr Leu Lys Pro Val Glu Lys Val Leu Lys
        355                 360                 365

Asp Ser Gly Leu Gln Lys Glu Asp Ile Asp Asp Ile Val Leu Val Gly
    370                 375                 380

Gly Ser Thr Arg Ile Pro Lys Val Gln Gln Leu Leu Glu Lys Phe Phe
385                 390                 395                 400

Asn Gly Lys Lys Ala Ser Lys Gly Ile Asn Pro Asp Glu Ala Val Ala
                405                 410                 415

Tyr Gly Ala Ala Val Gln Ala Gly Val Leu Ser Gly Glu Glu Gly Val
            420                 425                 430

Glu Asp Ile Val Leu Leu Asp Val Asn Ala Leu Thr Leu Gly Ile Glu
        435                 440                 445

Thr Thr Gly Gly Val Met Thr Pro Leu Ile Lys Arg Asn Thr Ala Ile
    450                 455                 460

Pro Thr Lys Lys Ser Gln Ile Phe Ser Thr Ala Val Asp Asn Gln Lys
465                 470                 475                 480

Ala Val Arg Ile Gln Val Tyr Glu Gly Glu Arg Ala Met Val Lys Asp
                485                 490                 495

Asn Asn Leu Leu Gly Asn Phe Glu Leu Ser Asp Ile Arg Ala Ala Pro
            500                 505                 510

Arg Gly Val Pro Gln Ile Glu Val Thr Phe Ala Leu Asp Ala Asn Gly
        515                 520                 525

Ile Leu Thr Val Ser Ala Thr Asp Lys Asp Thr Gly Lys Ser Glu Ser
    530                 535                 540

Ile Thr Ile Ala Asn Asp Lys Gly Arg Leu Ser Gln Asp Asp Ile Asp
545                 550                 555                 560

Arg Met Val Glu Glu Ala Glu Lys Tyr Ala Ala Glu Asp Ala Lys Phe
                565                 570                 575

Lys Ala Lys Ser Glu Ala Arg Asn Thr Phe Glu Asn Phe Val His Tyr
            580                 585                 590

Val Lys Asn Ser Val Asn Gly Glu Leu Ala Glu Ile Met Asp Glu Asp
        595                 600                 605
```

Asp Lys Glu Thr Val Leu Asp Asn Val Asn Glu Ser Leu Glu Trp Leu
610 615 620

Glu Asp Asn Ser Asp Val Ala Glu Ala Glu Asp Phe Glu Glu Lys Met
625 630 635 640

Ala Ser Phe Lys Glu Ser Val Glu Pro Ile Leu Ala Lys Ala Ser Ala
645 650 655

Ser Gln Gly Ser Thr Ser Gly Glu Gly Phe Glu Asp Glu Asp Asp Asp
660 665 670

Asp Tyr Phe Asp Asp Glu Leu
675

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2574 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 441..2429

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CACAATATCA ATAAGTTCCA CTCACGCTTT GTCTTTCACA ATATCATTTC AGAATTTACC 60

AATTTCGATT TTCATTGTTA CATTCATTGC TATGAAAACG TAAGGTGGTG GCGGCAATAG 120

GACTTATCGA AATGTACAGA ACTCACTATA GAATTGTTGT GTTGATGAGC TTCAACTGCA 180

TTCTTCTGGA AAGTACTAGT ATTAACGACG TGACTGCTCC TCTCGTTACT TAGCTGATTT 240

CTGGTACGCT ATTAAACTCA TCCAAAACCA ACTATTCTAG TTTGGTAAAT CTTAATCAAA 300

AACTATTAAA ACCCGTTTAC TATTTACTTA ACAGGTTGTT TTCAATAATT GGGAATTGCT 360

TGTGCCTACG ATCTCTTGTA ATTGAACTAC ACATATAAGC ATTTATAAGT TGGTAATCTT 420

CAAATTCTTG TTTATTGAAA ATG AAG AAG TTC CAG CTA TTT AGC ATT TTA 470
Met Lys Lys Phe Gln Leu Phe Ser Ile Leu
1 5 10

```
AGC TAC TTT GTA GCT TTA TTC CTC CTA CCT ATG GCT TTT GCT AGT GGT      518
Ser Tyr Phe Val Ala Leu Phe Leu Leu Pro Met Ala Phe Ala Ser Gly
                15                  20                  25

GAT GAT AAC TCT ACA GAA TCA TAT GGA ACA GTT ATT GGT ATT GAT CTT      566
Asp Asp Asn Ser Thr Glu Ser Tyr Gly Thr Val Ile Gly Ile Asp Leu
            30                  35                  40

GGT ACA ACA TAC TCT TGC GTT GCC GTT ATG AAA AAT GGT CGT GTA GAA      614
Gly Thr Thr Tyr Ser Cys Val Ala Val Met Lys Asn Gly Arg Val Glu
        45                  50                  55

ATT ATT GCC AAC GAT CAG GGT AAT CGT ATT ACA CCC TCA TAT GTG GCC      662
Ile Ile Ala Asn Asp Gln Gly Asn Arg Ile Thr Pro Ser Tyr Val Ala
    60                  65                  70

TTT ACT GAA GAC GAA CGT TTG GTT GGT GAG GCC GCT AAG AAC CAA GCT      710
Phe Thr Glu Asp Glu Arg Leu Val Gly Glu Ala Ala Lys Asn Gln Ala
75                  80                  85                  90

CCT TCC AAT CCT GAA AAC ACC ATT TTT GAC ATC AAG CGT CTT ATT GGA      758
Pro Ser Asn Pro Glu Asn Thr Ile Phe Asp Ile Lys Arg Leu Ile Gly
                95                  100                 105

CGT AAG TTT GAC GAA AAG ACA ATG GCC AAG GAT ATT AAA TCT TTT CCT      806
Arg Lys Phe Asp Glu Lys Thr Met Ala Lys Asp Ile Lys Ser Phe Pro
            110                 115                 120

TTC CAT ATT GTA AAT GAC AAG AAC CGT CCT TTG GTT GAG GTT AAT GTA      854
Phe His Ile Val Asn Asp Lys Asn Arg Pro Leu Val Glu Val Asn Val
        125                 130                 135

GGT GGT AAG AAG AAA AAG TTT ACC CCT GAA GAA ATT TCA GCC ATG ATT      902
Gly Gly Lys Lys Lys Lys Phe Thr Pro Glu Glu Ile Ser Ala Met Ile
    140                 145                 150

CTT AGT AAA ATG AAG CAA ACT GCT GAA GCT TAC CTC GGA AAG CCT GTC      950
Leu Ser Lys Met Lys Gln Thr Ala Glu Ala Tyr Leu Gly Lys Pro Val
155                 160                 165                 170

ACT CAC TCT GTT GTT ACT GTC CCC GCC TAC TTC AAT GAC GCT CAG CGT      998
Thr His Ser Val Val Thr Val Pro Ala Tyr Phe Asn Asp Ala Gln Arg
                175                 180                 185

CAG GCT ACC AAG GAT GCT GGT ACT ATT GCC GGC TTG AAT GTT ATT CGT     1046
Gln Ala Thr Lys Asp Ala Gly Thr Ile Ala Gly Leu Asn Val Ile Arg
            190                 195                 200

ATC GTC AAT GAG CCT ACT GCG GCT GCT ATT GCC TAC GGA TTA GAC AAA     1094
Ile Val Asn Glu Pro Thr Ala Ala Ala Ile Ala Tyr Gly Leu Asp Lys
        205                 210                 215
```

```
ACT GAT ACA GAG AAG CAT ATT GTT GTT TAT GAT TTA GGT GGT GGT ACT      1142
Thr Asp Thr Glu Lys His Ile Val Val Tyr Asp Leu Gly Gly Gly Thr
    220                 225                 230

TTT GAC GTT TCT CTT TTG TCT ATT GAC AAT GGT GTT TTC GAA GTT TTG      1190
Phe Asp Val Ser Leu Leu Ser Ile Asp Asn Gly Val Phe Glu Val Leu
235                 240                 245                 250

GCT ACT TCA GGT GAT ACC CAT CTC GGT GGT GAG GAC TTT GAC AAC CGT      1238
Ala Thr Ser Gly Asp Thr His Leu Gly Gly Glu Asp Phe Asp Asn Arg
                255                 260                 265

GTT ATC AAC TAC TTA GCC CGT ACT TAC AAC CGC AAG AAC AAT GTC GAT      1286
Val Ile Asn Tyr Leu Ala Arg Thr Tyr Asn Arg Lys Asn Asn Val Asp
            270                 275                 280

GTT ACT AAG GAT CTT AAG GCT ATG GGA AAA CTC AAG CGT GAA GTT GAA      1334
Val Thr Lys Asp Leu Lys Ala Met Gly Lys Leu Lys Arg Glu Val Glu
        285                 290                 295

AAA GCC AAC GGT ACT TTG TCC TCC CAA AAG TCT GTT CGT ATC GAG ATT      1382
Lys Ala Asn Gly Thr Leu Ser Ser Gln Lys Ser Val Arg Ile Glu Ile
    300                 305                 310

GAA TCT TTC TTT AAC GGT CAA GAC TTT TCT GAA ACT TTA TCC CGT GCT      1430
Glu Ser Phe Phe Asn Gly Gln Asp Phe Ser Glu Thr Leu Ser Arg Ala
315                 320                 325                 330

AAG TTC GAG GAG ATT AAA CAT GGA TCT CTT CAA GAA GAC TTT GAG CCT      1478
Lys Phe Glu Glu Ile Lys His Gly Ser Leu Gln Glu Asp Phe Glu Pro
                335                 340                 345

GTT GAG CAA GTA TTA AAG GAC TCC AAC CTC AAG AAA TCC GAG ATT GAT      1526
Val Glu Gln Val Leu Lys Asp Ser Asn Leu Lys Lys Ser Glu Ile Asp
            350                 355                 360

GAT ATC GTT CTT GTC GGT GGT TCT ACT CGT ATC CCT AAG GTT CAA GAA      1574
Asp Ile Val Leu Val Gly Gly Ser Thr Arg Ile Pro Lys Val Gln Glu
        365                 370                 375

CTT TTG GAG AGC TTC TTT GGT AAG AAG GCT TCT AAG GGT ATC AAT CCC      1622
Leu Leu Glu Ser Phe Phe Gly Lys Lys Ala Ser Lys Gly Ile Asn Pro
    380                 385                 390

GAT GAG GCT GTT GCC TAT GGT GCT GCT GTT CAA GCC GGC GTT TTA TCT      1670
Asp Glu Ala Val Ala Tyr Gly Ala Ala Val Gln Ala Gly Val Leu Ser
395                 400                 405                 410

GGC GAG GAA GGA AGT GAT AAC ATT GTC CTC TTG GAC GTT ATC CCT CTT      1718
Gly Glu Glu Gly Ser Asp Asn Ile Val Leu Leu Asp Val Ile Pro Leu
                415                 420                 425
```

```
ACT TTA GGT ATT GAG ACT ACC GGT GGT GTT ATG ACT AAA CTT ATC GGT      1766
Thr Leu Gly Ile Glu Thr Thr Gly Gly Val Met Thr Lys Leu Ile Gly
            430                 435                 440

CGT AAC ACT CCT ATT CCT ACT CGT AAG TCG CAA ATT TTC TCT ACT GCG      1814
Arg Asn Thr Pro Ile Pro Thr Arg Lys Ser Gln Ile Phe Ser Thr Ala
        445                 450                 455

GTT GAC AAT CAA AAT ACT GTT TTA ATT CAA GTC TAT GAA GGT GAA CGT      1862
Val Asp Asn Gln Asn Thr Val Leu Ile Gln Val Tyr Glu Gly Glu Arg
    460                 465                 470

ACT CTT ACT AAG GAC AAC AAC CTT CTT GGA AAA TTT GAC CTT CGT GGT      1910
Thr Leu Thr Lys Asp Asn Asn Leu Leu Gly Lys Phe Asp Leu Arg Gly
475                 480                 485                 490

ATT CCT CCT GCC CCT CGT GGT GTT CCC CAA ATT GAA GTC ACG TTT GAA      1958
Ile Pro Pro Ala Pro Arg Gly Val Pro Gln Ile Glu Val Thr Phe Glu
                495                 500                 505

GTC GAT GCC AAT GGT GTT TTG ACT GTT TCA GCC GTC GAC AAG TCT GGT      2006
Val Asp Ala Asn Gly Val Leu Thr Val Ser Ala Val Asp Lys Ser Gly
            510                 515                 520

AAG GGT AAG CCT GAG AAG CTT GTT ATC AAG AAT GAC AAA GGT CGT TTG      2054
Lys Gly Lys Pro Glu Lys Leu Val Ile Lys Asn Asp Lys Gly Arg Leu
        525                 530                 535

TCT GAG GAA GAT ATC GAG CGC ATG GTT AAG GAG GCC GAA GAA TTC GCT      2102
Ser Glu Glu Asp Ile Glu Arg Met Val Lys Glu Ala Glu Glu Phe Ala
    540                 545                 550

GAA GAA GAT AAG ATT TTG AAG GAG CGT ATT GAA GCT CGT AAT ACT CTT      2150
Glu Glu Asp Lys Ile Leu Lys Glu Arg Ile Glu Ala Arg Asn Thr Leu
555                 560                 565                 570

GAA AAC TAC GCC TAT TCT TTG AAA GGT CAA TTT GAC GAT GAT GAG CAA      2198
Glu Asn Tyr Ala Tyr Ser Leu Lys Gly Gln Phe Asp Asp Asp Glu Gln
                575                 580                 585

TTA GGT GGT AAG GTT GAT CCC GAA GAT AAG CAA GCT GTT TTG GAC GCT      2246
Leu Gly Gly Lys Val Asp Pro Glu Asp Lys Gln Ala Val Leu Asp Ala
            590                 595                 600

GTC GAA GAT GTT GCT GAA TGG CTT GAA ATC CAC GGA GAA GAT GCC AGC      2294
Val Glu Asp Val Ala Glu Trp Leu Glu Ile His Gly Glu Asp Ala Ser
        605                 610                 615

AAG GAA GAA TTT GAA GAT CAG CGT CAA AAA CTC GAT GCC GTT GTT CAT      2342
Lys Glu Glu Phe Glu Asp Gln Arg Gln Lys Leu Asp Ala Val Val His
    620                 625                 630
```

```
CCT ATT ACC CAA AAG TTG TAT TCC GAA GGA GCT GGT GAT GCT GAT GAA      2390
Pro Ile Thr Gln Lys Leu Tyr Ser Glu Gly Ala Gly Asp Ala Asp Glu
635                 640                 645                 650

GAG GAT GAT GAT TAC TTC GAT GAT GAG GCC GAT GAA CTT TAAAGTGTTT      2439
Glu Asp Asp Asp Tyr Phe Asp Asp Glu Ala Asp Glu Leu
                655                 660

TAAAATTGCC TGTACTTTCA TTTTTTAAGC TTTACTTAGT AATTTTTATT TAGTTCGAAG   2499

TATACGCAAG TCTGACTCGA ATGCTCTCAT GGTTTCATGA CCTTAATCTA AGGGTATTTG   2559

GAAACCAAAT GTTTT                                                    2574
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 663 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Lys Lys Phe Gln Leu Phe Ser Ile Leu Ser Tyr Phe Val Ala Leu
 1               5                  10                  15

Phe Leu Leu Pro Met Ala Phe Ala Ser Gly Asp Asp Asn Ser Thr Glu
            20                  25                  30

Ser Tyr Gly Thr Val Ile Gly Ile Asp Leu Gly Thr Thr Tyr Ser Cys
        35                  40                  45

Val Ala Val Met Lys Asn Gly Arg Val Glu Ile Ile Ala Asn Asp Gln
    50                  55                  60

Gly Asn Arg Ile Thr Pro Ser Tyr Val Ala Phe Thr Glu Asp Glu Arg
65                  70                  75                  80

Leu Val Gly Glu Ala Ala Lys Asn Gln Ala Pro Ser Asn Pro Glu Asn
                85                  90                  95

Thr Ile Phe Asp Ile Lys Arg Leu Ile Gly Arg Lys Phe Asp Glu Lys
            100                 105                 110

Thr Met Ala Lys Asp Ile Lys Ser Phe Pro Phe His Ile Val Asn Asp
        115                 120                 125
```

Lys Asn Arg Pro Leu Val Glu Val Asn Val Gly Gly Lys Lys Lys Lys
130 135 140

Phe Thr Pro Glu Glu Ile Ser Ala Met Ile Leu Ser Lys Met Lys Gln
145 150 155 160

Thr Ala Glu Ala Tyr Leu Gly Lys Pro Val Thr His Ser Val Val Thr
165 170 175

Val Pro Ala Tyr Phe Asn Asp Ala Gln Arg Gln Ala Thr Lys Asp Ala
180 185 190

Gly Thr Ile Ala Gly Leu Asn Val Ile Arg Ile Val Asn Glu Pro Thr
195 200 205

Ala Ala Ala Ile Ala Tyr Gly Leu Asp Lys Thr Asp Thr Glu Lys His
210 215 220

Ile Val Val Tyr Asp Leu Gly Gly Gly Thr Phe Asp Val Ser Leu Leu
225 230 235 240

Ser Ile Asp Asn Gly Val Phe Glu Val Leu Ala Thr Ser Gly Asp Thr
245 250 255

His Leu Gly Gly Glu Asp Phe Asp Asn Arg Val Ile Asn Tyr Leu Ala
260 265 270

Arg Thr Tyr Asn Arg Lys Asn Asn Val Asp Val Thr Lys Asp Leu Lys
275 280 285

Ala Met Gly Lys Leu Lys Arg Glu Val Glu Lys Ala Asn Gly Thr Leu
290 295 300

Ser Ser Gln Lys Ser Val Arg Ile Glu Ile Glu Ser Phe Phe Asn Gly
305 310 315 320

Gln Asp Phe Ser Glu Thr Leu Ser Arg Ala Lys Phe Glu Glu Ile Lys
325 330 335

His Gly Ser Leu Gln Glu Asp Phe Glu Pro Val Glu Gln Val Leu Lys
340 345 350

Asp Ser Asn Leu Lys Lys Ser Glu Ile Asp Asp Ile Val Leu Val Gly
355 360 365

Gly Ser Thr Arg Ile Pro Lys Val Gln Glu Leu Leu Glu Ser Phe Phe
370 375 380

Gly Lys Lys Ala Ser Lys Gly Ile Asn Pro Asp Glu Ala Val Ala Tyr
385 390 395 400

Gly Ala Ala Val Gln Ala Gly Val Leu Ser Gly Glu Glu Gly Ser Asp
405 410 415

Asn Ile Val Leu Leu Asp Val Ile Pro Leu Thr Leu Gly Ile Glu Thr
420 425 430

Thr Gly Gly Val Met Thr Lys Leu Ile Gly Arg Asn Thr Pro Ile Pro
435 440 445

Thr Arg Lys Ser Gln Ile Phe Ser Thr Ala Val Asp Asn Gln Asn Thr
450 455 460

Val Leu Ile Gln Val Tyr Glu Gly Glu Arg Thr Leu Thr Lys Asp Asn
465 470 475 480

Asn Leu Leu Gly Lys Phe Asp Leu Arg Gly Ile Pro Pro Ala Pro Arg
485 490 495

Gly Val Pro Gln Ile Glu Val Thr Phe Glu Val Asp Ala Asn Gly Val
500 505 510

Leu Thr Val Ser Ala Val Asp Lys Ser Gly Lys Gly Lys Pro Glu Lys
515 520 525

Leu Val Ile Lys Asn Asp Lys Gly Arg Leu Ser Glu Glu Asp Ile Glu
530 535 540

Arg Met Val Lys Glu Ala Glu Glu Phe Ala Glu Glu Asp Lys Ile Leu
545 550 555 560

Lys Glu Arg Ile Glu Ala Arg Asn Thr Leu Glu Asn Tyr Ala Tyr Ser
565 570 575

Leu Lys Gly Gln Phe Asp Asp Asp Glu Gln Leu Gly Gly Lys Val Asp
580 585 590

Pro Glu Asp Lys Gln Ala Val Leu Asp Ala Val Glu Asp Val Ala Glu
595 600 605

Trp Leu Glu Ile His Gly Glu Asp Ala Ser Lys Glu Glu Phe Glu Asp
610 615 620

Gln Arg Gln Lys Leu Asp Ala Val Val His Pro Ile Thr Gln Lys Leu
625 630 635 640

Tyr Ser Glu Gly Ala Gly Asp Ala Asp Glu Glu Asp Asp Asp Tyr Phe
645 650 655

Asp Asp Glu Ala Asp Glu Leu
660

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6030 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1004..4753

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTTTATCCTA TGTCACGGAC GACGACTTGT ATCACCTTGA ATTTTCTGAC CAAAGGGGCC 60

GAGTCGCTTC ACGAGGGGAT GAGAAAGGAA AAGAAGGGAA AACTAAACTT ATATAACGCA 120

GGTGTGTCTT TCTACCATTG CCATCAAGTT ATTAAAGGCC ACGAACAGGA ACGCTAGAGA 180

CCTGAGTTTG TCATTTGTTT AGTTCAAGGA TTAAATAAAC AATCCTTCTA CAAATAAGTC 240

CTTTCTTTCA CCATCGTCTT AAGACCACTG CCTCCAACGA AAACTAACCT AAAAGAGTTT 300

AGATCACGAG TATTTTCGCT CTTTCCCTCC TTCCCCTGGT TTTTTCTCGT TAGTTCTTTT 360

CATTTAAAAA CTCTTCTCTT GTCAAGAATT TAAAAGACGA AGAGTCCAAC ACCGACTGAT 420

TTTCTAACAG CAAAGGAACG AAGTTTTGCC GTGCAAACAA TAATTTCTAA ATTATAATTT 480

TGAGCCTAGC TGAGAAATAG GAGAGATTAT ATTTTAGAAA GGTAAGAAGT TTTTCTGTCA 540

TTCCTTTTAG AATATTTGCT ACGTTCTAAC ATTTTTTGTT ACTCAAGCGC ATTTTCTGCA 600

ACTTCCCTTA TAAGCTATTT CCTTTTTTTG GGACCGATCC TTTCTTCTGT CTTTGGTAAC 660

CTAAAAACCG GAATAGTCAA AGTTATCTGC ATAGTCTTCT TGCCAGGCTT ATTTTCGCCA 720

TACCATTTTT CTGGTACCCT AAACATTTTG GTCTTATTTT AGAACAGCTG GTGCCTCGTT 780

TTTCCGCATT AGGCGCACTT TTTTCATAGC CACTATTCTA AAAGAAACAA CTTTTTTTCA 840

AAGGGAAATC TAAGTTGCCT GCACGAAGAA TAAGACAAGG GTTCATAAAC GTATAGTATT 900

TGCCAAGTTC CATCTTTTTC TTTGTCACTT TAATATCGCA AAACAGAACA CCAAAAACCT 960

```
TTCAGCGCAA AGATTTGGCC CAATTATTCC ATCTTTATAC ACT ATG TCT AAA AAT      1015
                                                 Met Ser Lys Asn
                                                   1

AGC AAC GTT AAC AAC AAT AGA TCC CAA GAG CCA AAT AAC ATG TTT GTG      1063
Ser Asn Val Asn Asn Asn Arg Ser Gln Glu Pro Asn Asn Met Phe Val
  5                  10                  15                  20

CAA ACC ACA GGA GGT GGT AAA AAC GCC CCA AAG CAG ATT CAT GTT GCA      1111
Gln Thr Thr Gly Gly Gly Lys Asn Ala Pro Lys Gln Ile His Val Ala
                  25                  30                  35

CAC AGA CGT TCC CAA AGT GAG TTG ACA AAT TTG ATG ATT GAA CAA TTC      1159
His Arg Arg Ser Gln Ser Glu Leu Thr Asn Leu Met Ile Glu Gln Phe
             40                  45                  50

ACT TTG CAG AAG CAG TTG GAG CAA GTT CAA GCA CAG CAG CAA CAG TTG      1207
Thr Leu Gln Lys Gln Leu Glu Gln Val Gln Ala Gln Gln Gln Gln Leu
         55                  60                  65

ATG GCT CAG CAA CAG CAA TTG GCA CAA CAG ACA GGA CAA TAC CTG TCA      1255
Met Ala Gln Gln Gln Gln Leu Ala Gln Gln Thr Gly Gln Tyr Leu Ser
     70                  75                  80

GGA AAT TCT GGC TCT AAC AAT CAT TTC ACG CCT CAA CCG CCT CAC CCT      1303
Gly Asn Ser Gly Ser Asn Asn His Phe Thr Pro Gln Pro Pro His Pro
 85                  90                  95                 100

CAT TAC AAC TCA AAC GGT AAT TCA CCT GGT ATG AGT GCA GGT GGC AGC      1351
His Tyr Asn Ser Asn Gly Asn Ser Pro Gly Met Ser Ala Gly Gly Ser
                105                 110                 115

AGA AGT AGA ACT CAC TCC AGG AAC AAC TCC GGA TAT TAT CAT AAT TCA      1399
Arg Ser Arg Thr His Ser Arg Asn Asn Ser Gly Tyr Tyr His Asn Ser
            120                 125                 130

TAT GAT AAC AAT AAC AAT AGC AAT AAT CCT GGG TCT AAC TCA CAC AGA      1447
Tyr Asp Asn Asn Asn Asn Ser Asn Asn Pro Gly Ser Asn Ser His Arg
        135                 140                 145

AAG ACG AGT TCA CAA TCC AGC ATA TAT GGC CAT TCC AGA AGA CAT TCT      1495
Lys Thr Ser Ser Gln Ser Ser Ile Tyr Gly His Ser Arg Arg His Ser
    150                 155                 160

TTA GGT CTA AAT GAA GCG AAA AAG GCT GCT GCG GAA GAA CAA GCT AAA      1543
Leu Gly Leu Asn Glu Ala Lys Lys Ala Ala Ala Glu Glu Gln Ala Lys
165                 170                 175                 180
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AGA ATA TCT GGG GGT GAA GCA GGC GTA ACT GTG AAG ATA GAT TCT GTT      1591
Arg Ile Ser Gly Gly Glu Ala Gly Val Thr Val Lys Ile Asp Ser Val
                185                 190                 195

CAA GCT GAT AGT GGC TCA AAT TCT ACT ACA GAA CAA TCT GAT TTT AAA      1639
Gln Ala Asp Ser Gly Ser Asn Ser Thr Thr Glu Gln Ser Asp Phe Lys
            200                 205                 210

TTT CCA CCA CCA CCA AAT GCT CAT CAG GGC CAT CGT CGC GCA ACT TCA      1687
Phe Pro Pro Pro Pro Asn Ala His Gln Gly His Arg Arg Ala Thr Ser
        215                 220                 225

AAC CTA TCA CCT CCC TCT TTC AAA TTT CCT CCA AAC TCT CAC GGG GAT      1735
Asn Leu Ser Pro Pro Ser Phe Lys Phe Pro Pro Asn Ser His Gly Asp
    230                 235                 240

AAT GAC GAT GAA TTC ATA GCA ACC TCT TCA ACG CAC CGC CGT TCA AAG      1783
Asn Asp Asp Glu Phe Ile Ala Thr Ser Ser Thr His Arg Arg Ser Lys
245                 250                 255                 260

ACA AGA AAC AAT GAA TAT TCT CCA GGC ATT AAT TCC AAC TGG AGA AAC      1831
Thr Arg Asn Asn Glu Tyr Ser Pro Gly Ile Asn Ser Asn Trp Arg Asn
                265                 270                 275

CAA TCA CAG CAA CCT CAA CAG CAG CTT TCT CCA TTC CGC CAC AGA GGA      1879
Gln Ser Gln Gln Pro Gln Gln Gln Leu Ser Pro Phe Arg His Arg Gly
            280                 285                 290

TCT AAT TCA AGG GAT TAC AAT TCC TTC AAT ACC TTA GAA CCT CCT GCG      1927
Ser Asn Ser Arg Asp Tyr Asn Ser Phe Asn Thr Leu Glu Pro Pro Ala
        295                 300                 305

ATA TTT CAG CAG GGA CAC AAA CAT CGT GCC TCT AAT TCA TCA GTT CAT      1975
Ile Phe Gln Gln Gly His Lys His Arg Ala Ser Asn Ser Ser Val His
    310                 315                 320

AGT TTC AGT TCA CAA GGT AAT AAT AAC GGA GGT GGA CGT AAG TCC CTA      2023
Ser Phe Ser Ser Gln Gly Asn Asn Asn Gly Gly Gly Arg Lys Ser Leu
325                 330                 335                 340

TTT GCA CCC TAC CTT CCC CAA GCC AAC ATT CCA GAG CTA ATC CAA GAA      2071
Phe Ala Pro Tyr Leu Pro Gln Ala Asn Ile Pro Glu Leu Ile Gln Glu
                345                 350                 355

GGG AGA CTA GTA GCT GGT ATA TTA AGA GTT AAT AAA AAG AAT AGA TCG      2119
Gly Arg Leu Val Ala Gly Ile Leu Arg Val Asn Lys Lys Asn Arg Ser
            360                 365                 370

GAT GCC TGG GTC TCT ACA GAT GGC GCT CTT GAT GCG GAT ATT TAC ATT      2167
Asp Ala Trp Val Ser Thr Asp Gly Ala Leu Asp Ala Asp Ile Tyr Ile
        375                 380                 385
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TGC GGC TCC AAA GAT CGT AAT AGA GCA CTT GAA GGT GAT TTA GTC GCG      2215
Cys Gly Ser Lys Asp Arg Asn Arg Ala Leu Glu Gly Asp Leu Val Ala
    390                 395                 400

GTA GAA CTA TTA GTT GTG GAC GAT GTT TGG GAG TCC AAG AAA GAA AAG      2263
Val Glu Leu Leu Val Val Asp Asp Val Trp Glu Ser Lys Lys Glu Lys
405                 410                 415                 420

GAA GAA AAG AAG AGG AGA AAG GAT GCC TCT ATG CAA CAC GAT CTA ATT      2311
Glu Glu Lys Lys Arg Arg Lys Asp Ala Ser Met Gln His Asp Leu Ile
                425                 430                 435

CCT TTG AAC AGT AGT GAC GAT TAC CAC AAC GAT GCA TCT GTT ACT GCT      2359
Pro Leu Asn Ser Ser Asp Asp Tyr His Asn Asp Ala Ser Val Thr Ala
            440                 445                 450

GCA ACA AGC AAC AAT TTT CTA TCT TCT CCC TCC TCG TCT GAT TCG CTA      2407
Ala Thr Ser Asn Asn Phe Leu Ser Ser Pro Ser Ser Ser Asp Ser Leu
        455                 460                 465

AGC AAG GAT GAT TTA TCC GTC AGA AGA AAG AGG TCA TCT ACT ATC AAT      2455
Ser Lys Asp Asp Leu Ser Val Arg Arg Lys Arg Ser Ser Thr Ile Asn
    470                 475                 480

AAT GAT AGT GAT TCC TTA TCA TCT CCT ACC AAA TCA GGA GTA AGG AGA      2503
Asn Asp Ser Asp Ser Leu Ser Ser Pro Thr Lys Ser Gly Val Arg Arg
485                 490                 495                 500

AGA AGT TCA TTG AAA CAA CGT CCA ACT CAA AAG AAA AAT GAC GAT GTT      2551
Arg Ser Ser Leu Lys Gln Arg Pro Thr Gln Lys Lys Asn Asp Asp Val
                505                 510                 515

GAA GTT GAA GGT CAG TCA TTG TTA TTA GTT GAA GAA GAA GAA ATC AAC      2599
Glu Val Glu Gly Gln Ser Leu Leu Leu Val Glu Glu Glu Glu Ile Asn
            520                 525                 530

GAT AAA TAT AAG CCA CTT TAC GCA GGC CAT GTC GTT GCT GTT TTG GAC      2647
Asp Lys Tyr Lys Pro Leu Tyr Ala Gly His Val Val Ala Val Leu Asp
        535                 540                 545

CGT ATC CCT GGT CAG TTA TTT AGC GGT ACA TTA GGT TTG TTG AGA CCA      2695
Arg Ile Pro Gly Gln Leu Phe Ser Gly Thr Leu Gly Leu Leu Arg Pro
    550                 555                 560

TCC CAA CAA GCT AAT AGC GAC AAT AAC AAA CCA CCA CAA AGC CCA AAA      2743
Ser Gln Gln Ala Asn Ser Asp Asn Asn Lys Pro Pro Gln Ser Pro Lys
565                 570                 575                 580

ATT GCT TGG TTC AAG CCT ACT GAT AAG AAG GTG CCA TTA ATT GCA ATT      2791
Ile Ala Trp Phe Lys Pro Thr Asp Lys Lys Val Pro Leu Ile Ala Ile
                585                 590                 595
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CCT ACA GAA TTA GCT CCA AAG GAC TTT GTT GAA AAC GCT GAT AAA TAC      2839
Pro Thr Glu Leu Ala Pro Lys Asp Phe Val Glu Asn Ala Asp Lys Tyr
            600                 605                 610

TCC GAA AAG TTA TTC GTT GCC TCT ATT AAA CGT TGG CCA ATC ACA TCT      2887
Ser Glu Lys Leu Phe Val Ala Ser Ile Lys Arg Trp Pro Ile Thr Ser
        615                 620                 625

TTG CAT CCA TTT GGT ATT TTA GTT TCC GAA CTT GGA GAT ATT CAC GAT      2935
Leu His Pro Phe Gly Ile Leu Val Ser Glu Leu Gly Asp Ile His Asp
    630                 635                 640

CCT GAT ACT GAA ATT GAT TCC ATT TTA AGG GAT AAC AAT TTT CTT TCG      2983
Pro Asp Thr Glu Ile Asp Ser Ile Leu Arg Asp Asn Asn Phe Leu Ser
645                 650                 655                 660

AAT GAA TAT TTG GAT CAA AAA AAT CCG CAA AAA GAA AAA CCA AGT TTT      3031
Asn Glu Tyr Leu Asp Gln Lys Asn Pro Gln Lys Glu Lys Pro Ser Phe
                665                 670                 675

CAG CCG CTA CCA TTA ACG GCT GAA AGT CTA GAA TAT AGG AGG AAT TTT      3079
Gln Pro Leu Pro Leu Thr Ala Glu Ser Leu Glu Tyr Arg Arg Asn Phe
            680                 685                 690

ACG GAC ACT AAT GAG TAC AAT ATC TTT GCA ATT TCC GAG CTT GGA TGG      3127
Thr Asp Thr Asn Glu Tyr Asn Ile Phe Ala Ile Ser Glu Leu Gly Trp
        695                 700                 705

GTG TCT GAA TTT GCC TTA CAT GTC AGG AAT AAC GGA AAT GGT ACC CTA      3175
Val Ser Glu Phe Ala Leu His Val Arg Asn Asn Gly Asn Gly Thr Leu
    710                 715                 720

GAG CTG GGT TGT CAT GTT GTT GAT GTG ACC AGC CAT ATT GAA GAA GGC      3223
Glu Leu Gly Cys His Val Val Asp Val Thr Ser His Ile Glu Glu Gly
725                 730                 735                 740

TCC TCT GTT GAT AGG CGT GCG AGA AAG AGG TCC TCT GCG GTG TTC ATG      3271
Ser Ser Val Asp Arg Arg Ala Arg Lys Arg Ser Ser Ala Val Phe Met
                745                 750                 755

CCA CAA AAA CTT GTC AAT TTA TTA CCA CAA TCG TTC AAC GAC GAA CTG      3319
Pro Gln Lys Leu Val Asn Leu Leu Pro Gln Ser Phe Asn Asp Glu Leu
            760                 765                 770

TCG TTG GCC CCT GGC AAG GAA TCA GCC ACG CTG TCG GTT GTT TAC ACT      3367
Ser Leu Ala Pro Gly Lys Glu Ser Ala Thr Leu Ser Val Val Tyr Thr
        775                 780                 785

CTA GAC TCA TCT ACT TTA AGG ATT AAA TCT ACT TGG GTA GGC GAA TCT      3415
Leu Asp Ser Ser Thr Leu Arg Ile Lys Ser Thr Trp Val Gly Glu Ser
    790                 795                 800
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ACA ATT TCC CCC TCA AAC ATC TTG TCT TTA GAA CAA TTA GAC GAA AAA      3463
Thr Ile Ser Pro Ser Asn Ile Leu Ser Leu Glu Gln Leu Asp Glu Lys
805                 810                 815                 820

TTA TCT ACT GGA AGT CCC ACT AGC TAC CTC TCT ACT GTA CAG GAA ATT      3511
Leu Ser Thr Gly Ser Pro Thr Ser Tyr Leu Ser Thr Val Gln Glu Ile
                825                 830                 835

GCT AGA TCA TTT TAT GCT AGA AGA ATA AAT GAT CCA GAA GCT ACA TTA      3559
Ala Arg Ser Phe Tyr Ala Arg Arg Ile Asn Asp Pro Glu Ala Thr Leu
            840                 845                 850

CTT CCC ACC CTG TCC TTA TTG GAA AGC TTG GAT GAC GAA AAA GTT AAG      3607
Leu Pro Thr Leu Ser Leu Leu Glu Ser Leu Asp Asp Glu Lys Val Lys
        855                 860                 865

GTT GAC TTG AAC ATC CTG GAT AGA ACT TTA GGC TTT GTT GTA ATT AAT      3655
Val Asp Leu Asn Ile Leu Asp Arg Thr Leu Gly Phe Val Val Ile Asn
    870                 875                 880

GAG ATT AAA AGA AAG GTC AAC TCC ACT GTT GCA GAG AAA ATT TAC ACC      3703
Glu Ile Lys Arg Lys Val Asn Ser Thr Val Ala Glu Lys Ile Tyr Thr
885                 890                 895                 900

AAA CTT GGT GAT CTA GCT CTT TTG AGA AGG CAG ATG CAA CCC ATT GCA      3751
Lys Leu Gly Asp Leu Ala Leu Leu Arg Arg Gln Met Gln Pro Ile Ala
                905                 910                 915

ACC AAG ATG GCG TCA TTT AGA AAG AAA ATT CAA AAT TTT GGT TAC AAT      3799
Thr Lys Met Ala Ser Phe Arg Lys Lys Ile Gln Asn Phe Gly Tyr Asn
            920                 925                 930

TTT GAT ACC AAT ACG GCG GAT GAA TTA ATC AAA GGG GTG CTA AAA ATT      3847
Phe Asp Thr Asn Thr Ala Asp Glu Leu Ile Lys Gly Val Leu Lys Ile
        935                 940                 945

AAA GAT GAC GAT GTT AGA GTC GGA ATT GAA ATT TTA CTG TTT AAA ACC      3895
Lys Asp Asp Asp Val Arg Val Gly Ile Glu Ile Leu Leu Phe Lys Thr
    950                 955                 960

ATG CCA AGA GCT AGA TAC TTT ATT GCT GGC AAA GTA GAC CCG GAC CAA      3943
Met Pro Arg Ala Arg Tyr Phe Ile Ala Gly Lys Val Asp Pro Asp Gln
965                 970                 975                 980

TAT GGG CAT TAT GCC TTG AAC CTA CCT ATC TAC ACA CAT TTC ACA GCG      3991
Tyr Gly His Tyr Ala Leu Asn Leu Pro Ile Tyr Thr His Phe Thr Ala
                985                 990                 995

CCA ATG AGA AGA TAC GCT GAT CAT GTC GTT CAT AGG CAA TTA AAG GCC      4039
Pro Met Arg Arg Tyr Ala Asp His Val Val His Arg Gln Leu Lys Ala
            1000                1005                1010
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GTT ATC CAC GAT ACT CCA TAC ACC GAA GAT ATG GAA GCT TTG AAG ATT      4087
Val Ile His Asp Thr Pro Tyr Thr Glu Asp Met Glu Ala Leu Lys Ile
        1015                1020                1025

ACC TCC GAA TAT TGT AAT TTT AAA AAG GAC TGT GCT TAT CAA GCA CAG      4135
Thr Ser Glu Tyr Cys Asn Phe Lys Lys Asp Cys Ala Tyr Gln Ala Gln
    1030                1035                1040

GAA CAA GCA ATT CAT CTA TTG TTG TGT AAA ACA ATC AAC GAC ATG GGA      4183
Glu Gln Ala Ile His Leu Leu Leu Cys Lys Thr Ile Asn Asp Met Gly
1045                1050                1055                1060

AAT ACT ACA GGA CAA TTA TTA ACA ATG GCT ACT GTC TTA CAA GTT TAC      4231
Asn Thr Thr Gly Gln Leu Leu Thr Met Ala Thr Val Leu Gln Val Tyr
                1065                1070                1075

GAG TCC TCC TTT GAT GTA TTT ATT CCA GAA TTT GGT ATT GAA AAG AGA      4279
Glu Ser Ser Phe Asp Val Phe Ile Pro Glu Phe Gly Ile Glu Lys Arg
            1080                1085                1090

GTT CAT GGA GAT CAA CTA CCT TTG ATC AAA GCT GAG TTT GAT GGT ACC      4327
Val His Gly Asp Gln Leu Pro Leu Ile Lys Ala Glu Phe Asp Gly Thr
        1095                1100                1105

AAT CGT GTC TTG GAA TTG CAT TGG CAG CCC GGC GTA GAT AGT GCA ACT      4375
Asn Arg Val Leu Glu Leu His Trp Gln Pro Gly Val Asp Ser Ala Thr
    1110                1115                1120

TTT ATA CCA GCA GAT GAA AAA AAT CCA AAA TCC TAT AGA AAT TCC ATT      4423
Phe Ile Pro Ala Asp Glu Lys Asn Pro Lys Ser Tyr Arg Asn Ser Ile
1125                1130                1135                1140

AAG AAC AAA TTC AGA TCC ACA GCC GCT GAG ATT GCG AAT ATT GAA CTA      4471
Lys Asn Lys Phe Arg Ser Thr Ala Ala Glu Ile Ala Asn Ile Glu Leu
                1145                1150                1155

GAT AAA GAA GCG GAA TCT GAA CCA TTG ATC AGC GAT CCA TTG AGT AAG      4519
Asp Lys Glu Ala Glu Ser Glu Pro Leu Ile Ser Asp Pro Leu Ser Lys
            1160                1165                1170

GAA CTC AGC GAT TTG CAT CTA ACA GTA CCA AAT TTA AGG CTA CCA TCT      4567
Glu Leu Ser Asp Leu His Leu Thr Val Pro Asn Leu Arg Leu Pro Ser
        1175                1180                1185

GCA AGC GAC AAC AAG CAA AAT GCT TTA GAA AAA TTC ATT TCT ACT ACT      4615
Ala Ser Asp Asn Lys Gln Asn Ala Leu Glu Lys Phe Ile Ser Thr Thr
    1190                1195                1200

GAA ACC AGA ATT GAA AAT GAT AAC TAT ATA CAA GAA ATA CAT GAA TTG      4663
Glu Thr Arg Ile Glu Asn Asp Asn Tyr Ile Gln Glu Ile His Glu Leu
1205                1210                1215                1220
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CAA AAG ATT CCT ATT CTA TTG AGA GCT GAG GTG GGG ATG GCT TTG CCA 4711
Gln Lys Ile Pro Ile Leu Leu Arg Ala Glu Val Gly Met Ala Leu Pro
1225 1230 1235

TGT TTA ACC GTC CGT GCA TTA AAT CCA TTC ATG AAG AGG GTA 4753
Cys Leu Thr Val Arg Ala Leu Asn Pro Phe Met Lys Arg Val
1240 1245 1250

TAATCTCTTC TACCAATATC GTCATTGCTG TTTTTCTTGT TTTTCACTTT CGTTCTTTGG 4813

ATTGTGCTTC ACCCCTCAGT ATCCCTTCCC TTTGTTTTTA TTTCCTGCGA ACATTAACAA 4873

CTGCATGAAT TTTGTACTTC TCCTTTTAAT CCACGTTCCG GTAAGGCATC ATCCAAATTT 4933

TTTTATTCGA CCTCGTTAAG TCATATATTT TTTCCCAAAA ATACATAAAA CAATAATGCA 4993

GCCTTCTTTT CAATATTTAC AACTTTTCAA TTTATATTGT CTTTTGTTAT TTATACTCTT 5053

ATATATTAAA TTTATTCCGT TACTAAATAC CCTTTTGCTG TACAAATATC ATCAAAGAGA 5113

AGTACTGAAA GCTTACTTTT TATGCGCTGG GTAATTTTTC CGGAAACAAT AACGAAATCA 5173

TCGTCGAGCA ATTTTGCTCG TACTTCAGAA ACTACTGCGT AAACATTTGA GGTCGTACAA 5233

TAAGTAGATA GAAATAAATA AACCAATTTT TCGTCAGCGT TTAATCTGTA GCCAAAGATT 5293

TGTGGTATTC TCACAGTTTG AATAATATTC AGCTACTTCA TCAAGTAGTT TTTTTCAATA 5353

GGAGATTCAC GGTTCAATAA GTGCATTGAT TATGTTCGAC CAATTAGCAG TCTTTACCCC 5413

TCAAGGTCAA GTACTTTACC AATATAACTG TTTAGGAAAA AAGTTTTCTG AAATACAAAT 5473

TAACAGCTTT ATATCCCAGC TGATTACTTC CCCAGTAACT AGAAAAGAAA GTGTTGCAAA 5533

CGCAAATACA GACGGATTTG ATTTCAATCT TTTAACAATC AACAGCGAAC ACAAAAATTC 5593

TCCTTCATTT AATGCACTAT TTTATTTGAA TAAGCAACCA GAATTGTATT TCGTAGTGAC 5653

TTTTGCCGAG CAGACTTTAG AGCTTAATCA AGAAACTCAA CAAACACTTG CACTGGTGTT 5713

AAAACTCTGG AACTCATTGC ATTTAAGTGA ATCCATTCTA AAAAATCGTC AGGGCCAAAA 5773

CGAAAAGAAC AAGCATAACT ACGTCGATAT TCTTCAGGGA ATTGAAGACG ACCTGAAGAA 5833

ATTTGAGCAA TATTTTAGGA TAAAATATGA AGAGTCAATA AAACAAGACC ATATCAATCC 5893

AGATAATTTT ACCAAAAAATG GATCAGTACC CCAATCGCAT AATAAAAATA CCAAGAAAAA 5953

ATTGAGGGAT ACAAAAGGTA AGAAGCAATC TACAGGAAAT GTTGGTAGTG GGTAGTAAAG 6013

TGGGGCCGTG ATGGTGG 6030

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1250 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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Met Ser Lys Asn Ser Asn Val Asn Asn Asn Arg Ser Gln Glu Pro Asn
 1               5                  10                  15

Asn Met Phe Val Gln Thr Thr Gly Gly Gly Lys Asn Ala Pro Lys Gln
            20                  25                  30

Ile His Val Ala His Arg Arg Ser Gln Ser Glu Leu Thr Asn Leu Met
        35                  40                  45

Ile Glu Gln Phe Thr Leu Gln Lys Gln Leu Glu Gln Val Gln Ala Gln
    50                  55                  60

Gln Gln Gln Leu Met Ala Gln Gln Gln Gln Leu Ala Gln Gln Thr Gly
65                  70                  75                  80

Gln Tyr Leu Ser Gly Asn Ser Gly Ser Asn Asn His Phe Thr Pro Gln
                85                  90                  95

Pro Pro His Pro His Tyr Asn Ser Asn Gly Asn Ser Pro Gly Met Ser
            100                 105                 110

Ala Gly Gly Ser Arg Ser Arg Thr His Ser Arg Asn Asn Ser Gly Tyr
        115                 120                 125

Tyr His Asn Ser Tyr Asp Asn Asn Asn Asn Ser Asn Asn Pro Gly Ser
    130                 135                 140

Asn Ser His Arg Lys Thr Ser Ser Gln Ser Ser Ile Tyr Gly His Ser
145                 150                 155                 160

Arg Arg His Ser Leu Gly Leu Asn Glu Ala Lys Lys Ala Ala Ala Glu
                165                 170                 175

Glu Gln Ala Lys Arg Ile Ser Gly Gly Glu Ala Gly Val Thr Val Lys
            180                 185                 190

Ile Asp Ser Val Gln Ala Asp Ser Gly Ser Asn Ser Thr Thr Glu Gln
        195                 200                 205
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Ser Asp Phe Lys Phe Pro Pro Pro Pro Asn Ala His Gln Gly His Arg
210 215 220

Arg Ala Thr Ser Asn Leu Ser Pro Pro Ser Phe Lys Phe Pro Pro Asn
225 230 235 240

Ser His Gly Asp Asn Asp Asp Glu Phe Ile Ala Thr Ser Ser Thr His
245 250 255

Arg Arg Ser Lys Thr Arg Asn Asn Glu Tyr Ser Pro Gly Ile Asn Ser
260 265 270

Asn Trp Arg Asn Gln Ser Gln Gln Pro Gln Gln Gln Leu Ser Pro Phe
275 280 285

Arg His Arg Gly Ser Asn Ser Arg Asp Tyr Asn Ser Phe Asn Thr Leu
290 295 300

Glu Pro Pro Ala Ile Phe Gln Gln Gly His Lys His Arg Ala Ser Asn
305 310 315 320

Ser Ser Val His Ser Phe Ser Ser Gln Gly Asn Asn Asn Gly Gly Gly
325 330 335

Arg Lys Ser Leu Phe Ala Pro Tyr Leu Pro Gln Ala Asn Ile Pro Glu
340 345 350

Leu Ile Gln Glu Gly Arg Leu Val Ala Gly Ile Leu Arg Val Asn Lys
355 360 365

Lys Asn Arg Ser Asp Ala Trp Val Ser Thr Asp Gly Ala Leu Asp Ala
370 375 380

Asp Ile Tyr Ile Cys Gly Ser Lys Asp Arg Asn Arg Ala Leu Glu Gly
385 390 395 400

Asp Leu Val Ala Val Glu Leu Leu Val Val Asp Asp Val Trp Glu Ser
405 410 415

Lys Lys Glu Lys Glu Glu Lys Lys Arg Arg Lys Asp Ala Ser Met Gln
420 425 430

His Asp Leu Ile Pro Leu Asn Ser Ser Asp Asp Tyr His Asn Asp Ala
435 440 445

Ser Val Thr Ala Ala Thr Ser Asn Asn Phe Leu Ser Ser Pro Ser Ser
450 455 460

Ser Asp Ser Leu Ser Lys Asp Asp Leu Ser Val Arg Arg Lys Arg Ser
465 470 475 480

Ser Thr Ile Asn Asn Asp Ser Asp Ser Leu Ser Ser Pro Thr Lys Ser
485 490 495

Gly Val Arg Arg Arg Ser Ser Leu Lys Gln Arg Pro Thr Gln Lys Lys
500 505 510

Asn Asp Asp Val Glu Val Glu Gly Gln Ser Leu Leu Leu Val Glu Glu
515 520 525

Glu Glu Ile Asn Asp Lys Tyr Lys Pro Leu Tyr Ala Gly His Val Val
530 535 540

Ala Val Leu Asp Arg Ile Pro Gly Gln Leu Phe Ser Gly Thr Leu Gly
545 550 555 560

Leu Leu Arg Pro Ser Gln Gln Ala Asn Ser Asp Asn Asn Lys Pro Pro
565 570 575

Gln Ser Pro Lys Ile Ala Trp Phe Lys Pro Thr Asp Lys Lys Val Pro
580 585 590

Leu Ile Ala Ile Pro Thr Glu Leu Ala Pro Lys Asp Phe Val Glu Asn
595 600 605

Ala Asp Lys Tyr Ser Glu Lys Leu Phe Val Ala Ser Ile Lys Arg Trp
610 615 620

Pro Ile Thr Ser Leu His Pro Phe Gly Ile Leu Val Ser Glu Leu Gly
625 630 635 640

Asp Ile His Asp Pro Asp Thr Glu Ile Asp Ser Ile Leu Arg Asp Asn
645 650 655

Asn Phe Leu Ser Asn Glu Tyr Leu Asp Gln Lys Asn Pro Gln Lys Glu
660 665 670

Lys Pro Ser Phe Gln Pro Leu Pro Leu Thr Ala Glu Ser Leu Glu Tyr
675 680 685

Arg Arg Asn Phe Thr Asp Thr Asn Glu Tyr Asn Ile Phe Ala Ile Ser
690 695 700

Glu Leu Gly Trp Val Ser Glu Phe Ala Leu His Val Arg Asn Asn Gly
705 710 715 720

Asn Gly Thr Leu Glu Leu Gly Cys His Val Val Asp Val Thr Ser His
725 730 735

Ile Glu Glu Gly Ser Ser Val Asp Arg Arg Ala Arg Lys Arg Ser Ser
740 745 750

Ala Val Phe Met Pro Gln Lys Leu Val Asn Leu Leu Pro Gln Ser Phe
755 760 765

Asn Asp Glu Leu Ser Leu Ala Pro Gly Lys Glu Ser Ala Thr Leu Ser
770 775 780

Val Val Tyr Thr Leu Asp Ser Ser Thr Leu Arg Ile Lys Ser Thr Trp
785 790 795 800

Val Gly Glu Ser Thr Ile Ser Pro Ser Asn Ile Leu Ser Leu Glu Gln
805 810 815

Leu Asp Glu Lys Leu Ser Thr Gly Ser Pro Thr Ser Tyr Leu Ser Thr
820 825 830

Val Gln Glu Ile Ala Arg Ser Phe Tyr Ala Arg Arg Ile Asn Asp Pro
835 840 845

Glu Ala Thr Leu Leu Pro Thr Leu Ser Leu Leu Glu Ser Leu Asp Asp
850 855 860

Glu Lys Val Lys Val Asp Leu Asn Ile Leu Asp Arg Thr Leu Gly Phe
865 870 875 880

Val Val Ile Asn Glu Ile Lys Arg Lys Val Asn Ser Thr Val Ala Glu
885 890 895

Lys Ile Tyr Thr Lys Leu Gly Asp Leu Ala Leu Leu Arg Arg Gln Met
900 905 910

Gln Pro Ile Ala Thr Lys Met Ala Ser Phe Arg Lys Lys Ile Gln Asn
915 920 925

Phe Gly Tyr Asn Phe Asp Thr Asn Thr Ala Asp Glu Leu Ile Lys Gly
930 935 940

Val Leu Lys Ile Lys Asp Asp Asp Val Arg Val Gly Ile Glu Ile Leu
945 950 955 960

Leu Phe Lys Thr Met Pro Arg Ala Arg Tyr Phe Ile Ala Gly Lys Val
965 970 975

Asp Pro Asp Gln Tyr Gly His Tyr Ala Leu Asn Leu Pro Ile Tyr Thr
980 985 990

His Phe Thr Ala Pro Met Arg Arg Tyr Ala Asp His Val Val His Arg
995 1000 1005

Gln Leu Lys Ala Val Ile His Asp Thr Pro Tyr Thr Glu Asp Met Glu
1010 1015 1020

Ala Leu Lys Ile Thr Ser Glu Tyr Cys Asn Phe Lys Lys Asp Cys Ala
1025 1030 1035 1040

Tyr Gln Ala Gln Glu Gln Ala Ile His Leu Leu Leu Cys Lys Thr Ile
1045 1050 1055

Asn Asp Met Gly Asn Thr Thr Gly Gln Leu Leu Thr Met Ala Thr Val
1060 1065 1070

Leu Gln Val Tyr Glu Ser Ser Phe Asp Val Phe Ile Pro Glu Phe Gly
1075 1080 1085

Ile Glu Lys Arg Val His Gly Asp Gln Leu Pro Leu Ile Lys Ala Glu
1090 1095 1100

Phe Asp Gly Thr Asn Arg Val Leu Glu Leu His Trp Gln Pro Gly Val
1105 1110 1115 1120

Asp Ser Ala Thr Phe Ile Pro Ala Asp Glu Lys Asn Pro Lys Ser Tyr
1125 1130 1135

Arg Asn Ser Ile Lys Asn Lys Phe Arg Ser Thr Ala Ala Glu Ile Ala
1140 1145 1150

Asn Ile Glu Leu Asp Lys Glu Ala Glu Ser Glu Pro Leu Ile Ser Asp
1155 1160 1165

Pro Leu Ser Lys Glu Leu Ser Asp Leu His Leu Thr Val Pro Asn Leu
1170 1175 1180

Arg Leu Pro Ser Ala Ser Asp Asn Lys Gln Asn Ala Leu Glu Lys Phe
1185 1190 1195 1200

Ile Ser Thr Thr Glu Thr Arg Ile Glu Asn Asp Asn Tyr Ile Gln Glu
1205 1210 1215

Ile His Glu Leu Gln Lys Ile Pro Ile Leu Leu Arg Ala Glu Val Gly
1220 1225 1230

Met Ala Leu Pro Cys Leu Thr Val Arg Ala Leu Asn Pro Phe Met Lys
1235 1240 1245

Arg Val
1250

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 168 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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Ile Pro Pro Ala Pro Arg Gly Val Pro Gln Ile Glu Val Thr Phe Glu
1               5                  10                  15

Ile Asp Val Asn Gly Ile Leu Arg Val Thr Ala Glu Asp Lys Gly Thr
            20                  25                  30

Gly Asn Lys Asn Lys Ile Thr Ile Thr Asn Asp Gln Asn Arg Leu Thr
        35                  40                  45

Pro Glu Glu Ile Glu Arg Met Val Asn Asp Ala Glu Lys Phe Ala Glu
    50                  55                  60

Glu Asp Lys Lys Leu Lys Glu Arg Ile Asp Thr Arg Asn Glu Leu Glu
65                  70                  75                  80

Ser Tyr Ala Tyr Ser Leu Lys Asn Gln Ile Gly Asp Lys Glu Lys Leu
                85                  90                  95

Gly Gly Lys Leu Ser Ser Glu Gly Lys Glu Thr Met Glu Lys Ala Val
            100                 105                 110

Glu Glu Lys Ile Glu Trp Leu Glu Ser His Gln Asp Ala Asp Ile Glu
        115                 120                 125

Asp Phe Lys Ala Lys Lys Lys Glu Leu Glu Glu Ile Val Gln Pro Ile
    130                 135                 140

Ile Ser Lys Leu Tyr Gly Ser Gly Gly Pro Pro Pro Thr Gly Glu Glu
145                 150                 155                 160

Asp Thr Ser Glu Lys Asp Glu Leu
                165
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 654 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Lys Phe Pro Met Val Ala Ala Ala Leu Leu Leu Leu Cys Ala Val
1 5 10 15

Arg Ala Glu Glu Glu Asp Lys Lys Glu Asp Val Gly Thr Val Val Gly
20 25 30

Ile Asp Leu Gly Thr Thr Tyr Ser Cys Val Gly Val Phe Lys Asn Gly
35 40 45

Arg Val Glu Ile Ile Ala Asn Asp Gln Gly Asn Arg Ile Thr Pro Ser
50 55 60

Tyr Val Ala Phe Thr Pro Glu Gly Glu Arg Leu Ile Gly Asp Ala Ala
65 70 75 80

Lys Asn Gln Leu Thr Ser Asn Pro Glu Asn Thr Val Phe Asp Ala Lys
85 90 95

Arg Leu Ile Gly Arg Thr Trp Asn Asp Pro Ser Val Gln Gln Asp Ile
100 105 110

Lys Phe Leu Pro Phe Lys Val Val Glu Lys Lys Thr Lys Pro Tyr Ile
115 120 125

Gln Val Asp Ile Gly Gly Gly Gln Thr Lys Thr Phe Ala Pro Glu Glu
130 135 140

Ile Ser Ala Met Val Leu Thr Lys Met Lys Glu Thr Ala Glu Ala Tyr
145 150 155 160

Leu Gly Lys Lys Val Thr His Ala Val Val Thr Val Pro Ala Tyr Phe
165 170 175

Asn Asp Ala Gln Arg Gln Ala Thr Lys Asp Ala Gly Thr Ile Ala Gly
180 185 190

Leu Asn Val Met Arg Ile Ile Asn Glu Pro Thr Ala Ala Ala Ile Ala
195 200 205

Tyr Gly Leu Asp Lys Arg Glu Gly Glu Lys Asn Ile Leu Val Phe Asp
210 215 220

Leu Gly Gly Gly Thr Phe Asp Val Ser Leu Leu Thr Ile Asp Asn Gly
225 230 235 240

Val Phe Glu Val Val Ala Thr Asn Gly Asp Thr His Leu Gly Gly Glu
245 250 255

Asp Phe Asp Gln Arg Val Met Glu His Phe Ile Lys Leu Tyr Lys Lys
260 265 270

Lys Thr Gly Lys Asp Val Arg Lys Asp Asn Arg Ala Val Gln Lys Leu
275 280 285

Arg Arg Glu Val Glu Lys Ala Lys Arg Ala Leu Ser Ser Gln His Gln
290 295 300

Ala Arg Ile Glu Ile Glu Ser Phe Phe Glu Gly Glu Asp Phe Ser Glu
305 310 315 320

Thr Leu Thr Arg Ala Lys Phe Glu Glu Leu Asn Met Asp Leu Phe Arg
325 330 335

Ser Thr Met Lys Pro Val Gln Lys Val Leu Glu Asp Ser Asp Leu Lys
340 345 350

Lys Ser Asp Ile Asp Glu Ile Val Leu Val Gly Gly Ser Thr Arg Ile
355 360 365

Pro Lys Ile Gln Gln Leu Val Lys Glu Phe Phe Asn Gly Lys Glu Pro
370 375 380

Ser Arg Gly Ile Asn Pro Asp Glu Ala Val Ala Tyr Gly Ala Ala Val
385 390 395 400

Gln Ala Gly Val Leu Ser Gly Asp Gln Asp Thr Gly Asp Leu Val Leu
405 410 415

Leu Asp Val Cys Pro Leu Thr Leu Gly Ile Glu Thr Val Gly Gly Val
420 425 430

Met Thr Lys Leu Ile Pro Arg Asn Thr Val Val Pro Thr Lys Lys Ser
435 440 445

Gln Ile Phe Ser Thr Ala Ser Asp Asn Gln Pro Thr Val Thr Ile Lys
450 455 460

Val Tyr Glu Gly Glu Arg Pro Leu Thr Lys Asp Asn His Leu Leu Gly
465 470 475 480

Thr Phe Asp Leu Thr Gly Ile Pro Pro Ala Pro Arg Gly Val Pro Gln
485 490 495

Ile Glu Val Thr Phe Glu Ile Asp Val Asn Gly Ile Leu Arg Val Thr
500 505 510

Ala Glu Asp Lys Gly Thr Gly Asn Lys Asn Lys Ile Thr Ile Thr Asn
515 520 525

Asp Gln Asn Arg Leu Thr Pro Glu Glu Ile Glu Arg Met Val Asn Asp
530 535 540

Ala Glu Lys Phe Ala Glu Glu Asp Lys Lys Leu Lys Glu Arg Ile Asp
545 550 555 560

Thr Arg Asn Glu Leu Glu Ser Tyr Ala Tyr Ser Leu Lys Asn Gln Ile
565 570 575

Gly Asp Lys Glu Lys Leu Gly Gly Lys Leu Ser Ser Glu Asp Lys Glu
580 585 590

Thr Met Glu Lys Ala Val Glu Glu Lys Ile Glu Trp Leu Glu Ser His
595 600 605

Gln Asp Ala Asp Ile Glu Asp Phe Lys Ala Lys Lys Lys Glu Leu Glu
610 615 620

Glu Ile Val Gln Pro Ile Ile Ser Lys Leu Tyr Gly Ser Ala Gly Pro
625 630 635 640

Pro Pro Thr Gly Glu Glu Asp Thr Ser Glu Lys Asp Glu Leu
645 650

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5470 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 593..715

(ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 806..1036

(ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 1402..1539

(ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 2175..2289

(ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 2378..2764

(ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 2878..3115

(ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 3400..3568

(ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 4535..5095

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCCGGGGTCA CTCCTGCTGG ACCTACTCCG ACCCCCTAGG CCGGGAGTGA AGGCGGGACT 60

TGTGCGGTTA CCAGCGGAAA TGCCTCGGGG TCAGAAGTCG CAGGAGAGAT AGACAGCTGC 120

TGAACCAATG GGACCAGCGG ATGGGGCGGA TGTTATCTAC CATTGGTGAA CGTTAGAAAC 180

GAATAGCAGC CAATGAATCA GCTGGGGGGG CGGAGCAGTG ACGTTTATTG CGGAGGGGGC 240

CGCTTCGAAT CGGCGGCGGC CAGCTTGGTG GCCTGGGCCA ATGAACGGCC TCCAACGAGC 300

AGGGCCTTCA CCAATCGGCG GCCTCCACGA CGGGGCTGGG GGAGGGTATA TAAGCCGAGT 360

AGGCGACGGT GAGGTCGACG CCGGCCAAGA CAGCACAGAC AGATTGACCT ATTGGGGTGT 420

TTCGCGAGTG TGAGAGGGAA GCGCCGCGGC CTGTATTTCT AGACCTGCCC TTCGCCTGGT 480

TCGTGGCGCC TTGTGACCCC GGGCCCCTGC CGCCTGCAAG TCGAAATTGC GCTGTGCTCC 540

TGTGCTACGG CCTGTGGCTG GACTGCCTGC TGCTGCCCAA CTGGCTGGCA AGATGAAGCT 600

CTCCCTGGTG GCCGCGATGC TGCTGCTGCT CAGCGCGGCG CGGGCCGAGG AGGAGGACAA 660

GAAGGAGGAC GTGGGCACGG TGGTCGGCAT CGACTTGGGG ACCACCTACT CCTGGTAAGT 720

GGGGTTGCGG ATGAGGGGGA CGGGGCGTGG CGCTGGCTGG CGTGAGAAGT GCGGTGCTGA 780

TGTCCCTCTG TCGGGTTTTT GCAGCGTCGG CGTGTTCAAG AACGGCCGCG TGGAGATCAT 840

CGCCAACGAT CAGGGCAACC GCATCACGCC GTCCTATGTC GCCTTCACTC CTGAAGGGGA 900

ACGTCTGATT GGCGATGCCG CCAAGAACCA GCTCACCTCC AACCCCGAGA ACACGGTCTT 960

TGACGCCAAG CGGCTCATCG GCCGCACGTG GAATGACCCG TCTGTGCAGC AGGACATCAA 1020

GTTCTTGCCG TTCAAGGTTC GACCGGTTTT CCTCATCCAG TTAGAGAACG GGTGGGTGGT 1080

GGGAGTATTT AGAGTTATAA GTCTCTGGAA AAGTGTTGAG ACAACAGTTG AAGGTTATAG 1140

ACATGATGTA TGTAATAACT TTAATACTAT TAGTATGTTA CAAAACTTAA GACAGTTGCT 1200

GTCGTACTGT CTACGATAGT TTAGGAATAA AAGACCGATT AAAACTGAAC TTTGTAAGAC 1260

ACCTATACTC CCTGAAGTAT TTCTAGTCAA TTTGCAGCCC CAAGGGACCA AAATAAACCA 1320

AATTGTGGGG ATGGTAGTGG GTCTTTTAAA CTTTGAGATG TCATTGTATC TGTGTCTGAA 1380

AACAATAATT CTTTAAAATA GGTGGTTGAA AAGAAAACTA AACCATACAT TCAAGTTGAT 1440

ATTGGAGGTG GGCAAACAAA GACATTTGCT CCTGAAGAAA TTTCTGCCAT GGTTCTCACT 1500

AAAATGAAAG AAACCGCTGA GGCTTATTTG GGAAAGAAGG TAAATATTTC TAGAACAATG 1560

TTAAGTATTT TTTGATCATT AGTATTCTCG GTTGGCTGTT ATGTATAGAA GCCTTCGTGA 1620

AGGGTTTCAA AAATTTTAAT CAGAATGGTA TTCATGCTTG TCACGGTTTA ATTATTGAGT 1680

CCCTTTACTA TAAGCCAAAC AAAAATAGAC TTTTCATGTA TTATTTAATG CTTACAATTC 1740

CAGGAACAAT AAAATTTTAT ATGTTGTATT CATCAATAAT TGGCTTAAAA ACTAAAGTGA 1800

TGGTTTGACT GTAATTTTTT TTTTTTGAGA TGGAGTCTTG CTCTGTTGCC CAGGCTGGAC 1860

TGCAGTGGCA CGATCTCAGC TCACTGCAAC CTCTGCCTCC CGGGTTAAGC AGCTCTCCTG 1920

CCTCAGCCTC CAAGTAATGG AACGACAGGC ACACCACCAC AGCTGGCTAA TTTTTTTTTT 1980

TTTTTTTAAT TTTCAGTAGA GACAGGGTTT CTCCACATTG CCAGGCTGGT CTTGAAATCC 2040

TGCCCTCAGG TTGATCCTCC TGCCTAGCCT CCCAAAGTGC TGGATTATAG GCAGAAGCCA 2100

CCGCCTGGCC AGACTGTAAT TTAAATAAGG GTTAAACTAT GTGACAATAC ACTTAATTAT 2160

CTTTATCCTT TTAGGTTACC CATGCAGTTG TTACTGTACC AGCCTATTTT AATGATGCCC 2220

AACGCCAAGC AACCAAAGAC GCTGGAACTA TTGCTGGCCT AAATGTTATG AGGATCATCA 2280

ACGAGCCGTA AGTATGAAAT TCAGGGATAC GGCATATTTG CCAAATAGTG GAAATGTGAA 2340

GTACTGACAA AACTTTTCCC TTTTTCAATC TAATAGTACG GCAGCTGCTA TTGCTTATGG 2400

CCTGGATAAG AGGGAGGGGG AGAAGAACAT CCTGGTGTTT GACCTGGGTG GCGGAACCTT 2460

CGATGTGTCT CTTCTCACCA TTGACAATGG TGTCTTCGAA GTTGTGGCCA CTAATGGAGA 2520